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WILLIAM TITUS HORNE
1876–1944

WILLIAM TITUS HORNE
1876–1944

T. E. Rawlins

We were very sorry to learn of the death of Professor William Titus Horne on April 12, 1944. In his passing his former students and colleagues have lost one of their finest friends.

Professor Horne was born November 8, 1876, in Kankakee, Illinois. He graduated from the University of Nebraska in 1898, and later had a year of graduate work at Columbia University. From 1904 to 1909 he was connected with the Agricultural Experiment Station of the Cuban Government, the last three years as Chief of the Division of Plant Pathology. In 1909 he joined the staff of the Plant Pathology Division of the University of California at Berkeley where he took over the teaching of plant pathology and continued this important work until 1928. During this period his keen interest in the welfare of his students encouraged many of them to take up plant pathology as a life work. While in Berkeley he and his students worked on a number of important and difficult problems. Among them were brown rot and virus diseases of stone fruits, strawberry virus diseases, olive diseases, Armillaria root rot and fig spoilage. Numerous important publications resulted from this work.

In 1928 Professor Horne moved to the Citrus Experiment Station at Riverside, California, where he began research on diseases of the avocado and certain other subtropical fruits, a field in which he had always been particularly interested. He was also very successful in this endeavor as is indicated by his being honored with one of the first service medals presented by the California Avocado Society in 1939 and by his election to the presidency of the Pacific Division of the American Phytopathological Society in 1938–39. During this period he made numerous contributions on the diseases of the avocado and other subtropical fruits. His bulletin, "Avocado Diseases in Southern California," is probably the most complete and reliable publication on avocado diseases and is a final excellent chapter in an outstanding life of service.

He was author or co-author of the following publications:

A new species of Lembosia (*Lembosia Rolfsii* sp. nov.). Bull. Torrey Bot. Club **32**: 69–71. 1905.

El Minero de las hojas y otras plagas del cafeto. (Coffee leaf miner and other coffee pests.) In English and Spanish. Estac. Cent. Agron. Cuba Bull. 3. 1905. (With Mel. T. Cook.)

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Pudricion del Cogollo y otras enfermedades del cocotero en Cuba. (The bud rot and some other cocoanut troubles in Cuba.) Estac. Cent. Agron. Cuba Bull. 15. 1908.

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The method of spreading of the olive knot disease. Phytopath. **2**: 101–105. 1912. (With Wm. B. Parker and Lyman L. Daines.)
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Prevention of wood decay in fruit trees. Better Fruit **9**: 35–37. 1914.
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Citrus blast and black pit. Calif. Citrograph **6**: 234. 1923. (With H. S. Fawcett and A. F. Camp.)
Avocado fruit decays. Calif. Avocado Assn., Rpt. **1925–1926**: 96–99. 1926.
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Notes on fruit decays of the feijoa (*Feijoa Sellowiana* Berg.) Year Book Calif. Avocado Assn., Rept. **1927**: 31–33. 1927.
Notes on the experimental inoculation of avocado seedlings with the pear blight organism, *Bacillus amylovorus* (Burr.) Trev. U. S. Dept. Agr. Plant Disease Reporter **12**: 7. 1928.
The improved eastern blueberry in California. Madrono **1**: 179–183. 1928.
Avocado anthracnose. Year Book Calif. Avocado Assn. **1929**: 12–14. 1929.
Carapace spots (avocado). Year Book Calif. Avocado Assn. **1929**: 129. 1929.
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A mosaic of the fig in California. Phytopath. **23**: 887–896. 1933. (With I. J. Condit.)
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THE INFLUENCE OF FUNGI ON STORAGE, ON SEED VIABILITY AND SEEDLING VIGOR OF SOYBEANS[1]

IAN W. TERVET

(Accepted for publication May 7, 1944)

Except for the information given by Dickson (2), there are few published reports on the occurrence of bacterial and fungus pathogens on soybean seed. Some fungi have been isolated from soybean meal, silage, or other products derived from soybeans. From moldy soybean cakes, species of *Aspergillus, Penicillium, Monilia,* and *Sphaerella* were obtained (7), while *Byssochlamys musticola* Naoumoff and Kiryalava was isolated from nonacid soybean silage (6). Ramstad and Geddes (8) report several fungi in soybeans damaged in storage, while Milner *et al.* (5) have shown that soybeans injured by frost have more seeds infected by fungi, principally *Alternaria* and *Fusarium,* than uninjured seeds.

Satisfactory storage of soybeans to avoid heating and spoilage requires, beside suitable storage conditions, that the beans be maintained at a low moisture content. Burlison *et al.* (1) have shown that deterioration of soybean seed in farm storage is most rapid when the seeds are allowed to absorb moisture, and potential germination decreases rapidly under such a condition. Ramstad and Geddes (8) also observed that the viability of soybeans decreased rapidly under conditions favoring the growth of microorganisms, and that soybeans suffered damage when stored at high moisture levels at room temperature, even though heating did not occur.

The microflora was studied in soybean seed grown in 19 counties of Minnesota in 1942. Studies on the relation of fungi to heating and to viability of soybeans also were initiated.

MICROFLORA OF FROST-INJURED SOYBEAN SEED

Much of the soybean seed harvested in Minnesota in 1942 was injured by frost, the extent of the injury on different samples varying greatly. Many samples were green and shrivelled, the germination of the seed being generally poor. Surface-disinfected soybean seeds[2] were plated on potato-dextrose agar. Frost-injured seeds were more likely to be infected by fungi and bacteria than noninjured seeds. *Alternaria* predominated, with *Fusarium* next in frequency on the seeds. *Aspergillus spp.* occurred on occa-

[1] Paper No. 2161, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul.

Cooperative investigations between the Division of Plant Pathology and Botany and Agricultural Biochemistry. The writer expresses his deep appreciation to Dr. W. F. Geddes and Mr. Max Milner, of the Division of Agricultural Biochemistry, for preparing many of the soybean samples, for supplying all of the moisture data as well as the results obtained in the adiabatic studies given in this report, and for their interest and enthusiasm during the course of the work.

[2] Seeds dipped in 70 per cent alcohol, immersed in mercuric chloride (1: 1000) for 2 min., washed in water for 5 min., rinsed in calcium hypochlorite 1.3 per cent for not less than 5 min., then plated directly from the latter solution on agar plates.

sional samples, in one case 25 per cent of the seeds being infected. *Rhizopus nigricans* Ehr., *Chaetomium sp.*, *Cephalothecium roseum* Corda, *Trichoderma viride* (Pers.) Fr., and some nonsporulating fungi were found. Bacteria were present in most severely shrivelled samples and not infrequently developed from seeds that yielded also *Alternaria* and *Fusarium*. Indeed, it was not uncommon for seeds to give rise to two different colonies of fungi, or a fungus and bacterium. A summary of the microflora analysis is in table 1, the seed samples being grouped on a regional basis.

Two series of soybean samples combining seed lots with different percentages of frost-injured seeds were analyzed for microflora and for the effect of seed treatment on the germination of the seeds. One set of samples was obtained from Illinois through the courtesy of the Regional Soybean Laboratory, Peoria, Illinois; the second sample was of Minnesota origin. The Illinois samples were less severely injured by frost, and on the average

TABLE 1.—*The occurrence of microorganisms on soybean seeds injured by frost*

Origin of samples	No. of samples	Percentage of soybean seeds infected by fungi and bacteria					Frost-injured beans (per cent)
		Sterile	*Alternaria*	*Fusarium*	Miscellaneous fungi	Bacteria	
South East Minnesota	45	Av. 35.9 Range 0–85	28.9 9–53	19.0 2–41	2.2 0–12	16.0 1–82	33.0 4–78
South West Minnesota	24	Av. 43.7 Range 2–88	33.2 1–71	12.1 0–44	1.6 0–25	14.3 0–65	22.3 0–58
Central Minnesota	11	Av. 24.0 Range 4–69	56.3 29–81	10.0 0–24	0 0	15.2 1–46	40.3 8–67

had fewer infected seeds. *Alternaria* was the most common fungus in the Minnesota samples, *Fusarium* being less abundant; in the Illinois samples *Alternaria* and *Fusarium* were found in equal amounts (Table 2). A nonsporulating fungus, resembling that mentioned by Johnson and Koehler (4), was commonly associated with a purple spotting on seeds from the Illinois samples. The number of seeds free from infection decreased as the percentage of frost-injured seeds increased. Germination of the seeds varied with the extent of frost injury, the lots with least injury germinating better than those with more injured seeds. Treatment of the seeds with maximum adhesive dosage of Arasan, 50 per cent tetramethylthiuram disulphide, consistently increased the total stand and the percentage of vigorous plants as measured by the number of seedlings with expanded first leaves.

THE MICROFLORA OF SOYBEANS IN RELATION TO LOSS IN VIABILITY AND HEATING OF SOYBEANS

Soybeans with a high moisture content heat rapidly in storage and at the same time the respiration of the sample also is accelerated. It has been suggested that microorganisms play an important part in the high respira-

tory rates associated with heating (8), but no detailed analysis of the microflora of high-moisture-content beans during storage is available. Attempts were made to determine the changes in microflora associated with increasing moisture content of the beans and with increasing storage temperatures.

The Minnesota lot of soybeans, with 55 per cent frost damage (Table 2), had a moisture content of 6.6 per cent after storage at 2° C. for 2 months. A sample of this lot of beans was conditioned to 17.6 per cent moisture content by placing the seeds in a humidifying chamber at room temperature for 4 weeks, the seeds then being stored at 2° C. for 5 days, and thereafter placed in a Dewar flask. The seeds were very moldy after 1 month's storage

TABLE 2.—*The microflora of frost-injured soybeans and the value of seed treatment in improving germination*

Seed source and frost damage (per cent)	Percentage seeds infected with fungi and bacteria					Seeds treated with Arasan		Seeds not treated	
	Sterile	*Alternaria*	*Fusarium*	Miscellaneous fungi	Bacteria	Seeds germinating	Plants with first leaves expanded	Seeds germinating	Plants with first leaves expanded
						Pct.	*Pct.*	*Pct.*	*Pct.*
Minnesota									
10.3	70	25	1	1[a]	3	62	94	54	89
13.3	77	18	5	0	0	51	94	41	88
21.0	50	30	8	1[a]	1	52	87	39	77
21.0	50	20	6	0	24	33	79	26	73
26.0	49	27	11	0	13	28	93	20	85
41.0	42	39	3	1[a]	15	23	87	14	71
55.0	27	40	8	2[a]	23	6	67	1	0
Illinois									
3.3	85	0	4	2[b]	9	81	97	75	95
6.4	93	6	1	0	0	80	92	73	96
7.5	77	6	0	2	15	69	91	63	92
10.5	56	10	0	2	32	59	92	48	90
12.3	84	1	15	0	0	60	87	44	84
18.0	57	12	25	0	6	46	87	31	84

[a] *Rhizopus sp., Chaetomium sp., Aspergillus sp.*
[b] Nonsporulating fungus.

in a humidifier. Two hundred surface-disinfected seeds were plated on potato-dextrose agar. Only 6 per cent of the seeds were free from internal fungi, as opposed to 27 per cent sterile seeds in the lot with a moisture content of 6.6 per cent. Also, 66 per cent of the high-moisture-content seeds gave rise to *Aspergillus spp.*, as opposed to 1 per cent in the low-moisture seeds, a very great increase in the prevalence of this fungus at the expense of other fungi and bacteria.

The seeds in the Dewar flasks were allowed to undergo adiabatic heating in an apparatus similar to that described by Ramstad and Geddes (8). In 21 days the temperature rose to 46.9° C. from an initial temperature of 22° C. The beans were then brown and free from superficial mold growth. These seeds were surface-disinfected and plated on potato-dextrose agar, and

98 per cent were free from internal fungi, while 2 per cent produced colonies of bacteria.

Samples of 1942 Illinois soybeans with an initial moisture content of 6.51 per cent were conditioned to a higher moisture level by placing them in a humidifier. When the seeds reached a moisture content of 8.65 per cent after 4 days in the humidifier a sample was removed and placed in a refrigerator at 2° C., where it remained for 6½ weeks, until the storage experiments were begun. The rest of the seeds were kept in the humidifier for 30 days, their moisture content at the end of that time being 12.95 per cent. These seeds were then kept at 2° C. for 3 weeks. A sample was removed, sprinkled with water, thoroughly shaken and replaced in the refrigerator for 2 days,

TABLE 3.—*The effect of adiabatic aerated storage on the microflora and viability of soybeans*

Moisture content of seeds	Storage temperature	No. days in adiabatic storage	Microflora of seeds			Seeds treated with Arasan		Seeds not treated	
			Sterile	*Aspergillus*	Misc. fungi[a]	Seeds germinating	Plants with first leaves expanded	Seeds germinating	Plants with first leaves expanded
Pct.	*Degrees, C.*					*Pct.*	*Pct.*	*Pct.*	*Pct.*
6.51	.....	0	97	0	3	95	92	93	76
8.65	.....	0	97	0	3	95	98	92	89
12.95	.....	0	91	4	5	85	82	71	11
21.2	.....	0	89	3	8	86	78	78	4
"	29.4	3	84	5	11	72	78	73	12
"	37.05	5	73	17	10	68	78	52	48
"	44.4	7	36	53	11	41	37	30	17
"	49.3	8	60	36	4	0	0	0	0
"	53.7	10	84	14	2	0	0	0	0
"	55.1	12	87	11	2	0	0	0	0
"	54.9	13	91	4	5	0	0	0	0
"	59.7	15	100	0	0	0	0	0	0
"	67.3	17	100	0	0	0	0	0	0
"	77.0	19	100	0	0	0	0	0	0

[a] Mainly *Chaetomium* sp., *Cephalothecium roseum*, *Rhizopus nigricans*, *Cunninghamella echinulata*, and *Penicillium* sp.

the final moisture content of the seeds of this sample being 21.2 per cent. Four samples with moisture levels varying from 6.51 to 21.2 per cent (Table 3) were obtained by these methods. Plating of 200 seeds of each of these samples on potato-dextrose agar, after surface disinfection, showed that the higher-moisture samples had slightly more infected seeds than the low-moisture samples.

Ten lots of beans with a moisture content of 21.2 per cent were placed in bottles of approximately 300 cc. capacity, about 100 g. of beans in each bottle. These samples were then placed in the adiabatic storage chamber. The bottles and flask were continuously aerated, approximately 8 liters of air per 24 hours at start of experiment, the air being passed through a humidifying solution in order to maintain the moisture content in the seeds.

At intervals (Table 3) bottles of soybeans were removed from storage and the beans immediately analyzed for microflora and tested for viability. Two hundred seeds were plated, and duplicate 100-seed rows of nontreated seeds and of seeds treated with the maximum-adhesion dose of Arasan were planted in sand in a greenhouse at 70° F. Stand counts were made 10 days after planting.

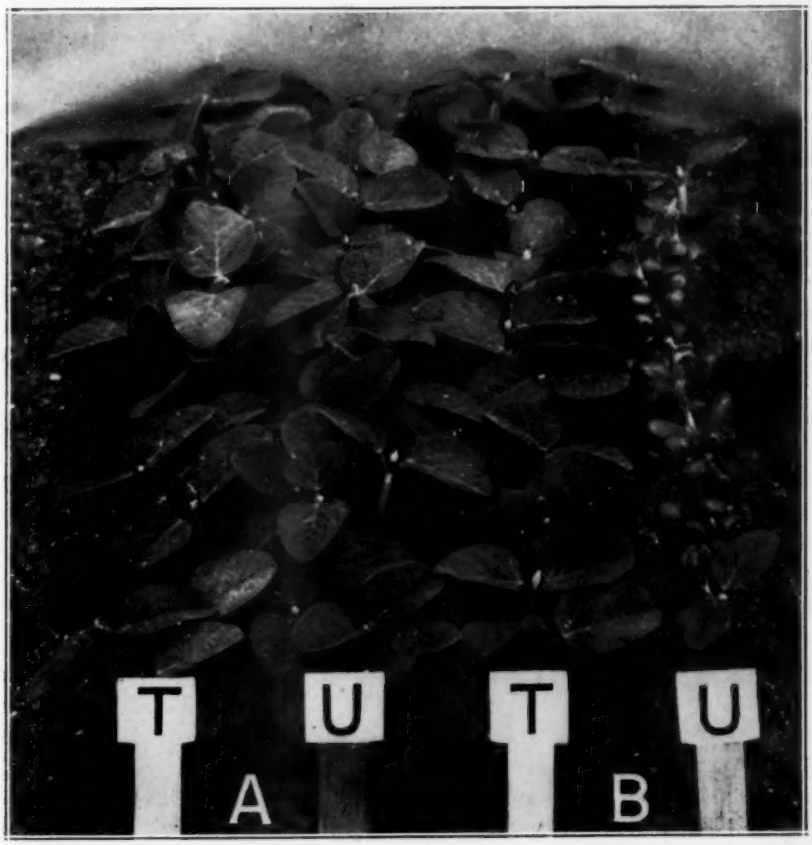

FIG. 1. Effect of seed treatment on stand and vigor of soybean seed lots. A. Plants from low-moisture-content seeds (6.51 per cent), treated with Arasan (T) and untreated (U). B. Plants from high-moisture-content seeds (21.2 per cent) treated with Arasan (T) and untreated (U).

The beans heated rapidly, a temperature of 37.05° C. being reached in 5 days, and 49.3° C. in 8 days, with a maximum of 77.0° C. after 19 days. During the first week in storage, the percentage of noninfected seeds decreased from 89 per cent at room temperature (± 22° C.) to 36 per cent at 44.4° C. Coincident with the rise in temperature, the number of seeds infected with *Aspergillus spp.* increased rapidly from 5 per cent at room temperature to a maximum of 53 per cent at 44.4° C. Beans sampled on the 8th day of storage, when the temperature had reached 49.3° C., had fewer

infected seeds; and by the 15th day at a temperature of 59.7° C. no fungi were found. At the favorable temperature, the predominant fungi were *Aspergillus* species, the following group species (9) being most common: *A. glaucus, A. flavus,* and *A. ochraceus,* while *A. niger* and *A. fumigatus* were less frequently found. Several other fungi were also present on the seeds—*Cephalothecium roseum* Corda, *Cunninghamella echinulata* Thaxter, *Rhizopus nigricans* Ehr., *Chaetomium sp.* and *Penicillium sp.*

The viability of seeds decreased with increased moisture content of the seed and with rising temperature in storage. Seeds with moisture contents of 6.51 and 8.65 per cent germinated above 90 per cent, with 76 and 89 per cent respectively of the seedlings producing their first leaves within 10 days of planting (Table 3). At a moisture content of 12.95 per cent, 71 per cent of the seeds germinated but only 11 per cent of the plants were vigorous. At 21.2 per cent moisture, 78 per cent of the seeds germinated, but only 4 per cent of the seedlings had produced first leaves 12 days after planting. Treatment of this high-moisture-content seed with the maximum-adhesion dose of Arasan increased the germination to 86 per cent and the number of seedlings with first leaves to 78 per cent (Fig. 1). The viability of seed stored in the adiabatic respirometer decreased rapidly, from 78 per cent at the beginning of storage to 30 per cent at the end of 7 days, and 0 per cent on the 8th day and thereafter. The seeds in storage generally gave rise to stunted plants, except one lot that had been stored for 5 days and in which 48 per cent of the seedlings were vigorous. The reasons for the occurrence of such a high proportion of vigorous plants in this single sample were not determined, but it is possible that the variability of results could be considered as experimental error, although the duplicated rows of seedlings were very similar.

In general, applications of the maximum-adhesion dosage of Arasan to the seeds just prior to planting increased the total stand and resulted in a marked decrease in the percentage of stunted plants, indication that the poor development of the seedlings was due to the presence of microorganisms on the seed (Table 3 and Fig. 1).

EFFECT OF HIGH MOISTURE CONTENT ON THE MICROFLORA AND VIABILITY OF SOYBEANS

Three samples of 1942 Minsoy soybeans were conditioned to different moisture contents by adding water to the air-dried beans, thereafter frequently shaking the samples. The beans were stored at room temperature in quart jars with tight-fitting lids, each jar containing 1 lb. of seeds. The moisture contents of the beans after conditioning were 7.9, 13.3, and 20.4 per cent, respectively.

Six days after the water had been added, 200 seeds of each lot of beans were plated on potato-dextrose agar after surface disinfection, and duplicated 100-seed rows of seed from each sample, untreated and treated with the maximum-adhesion load of Arasan, were planted in sand in a greenhouse

at 70° F. Similar tests were made 13, 25, and 34 days after the initiation of the experiment. The low moisture sample remained fairly constant throughout the storage period, no major changes occurring in the microflora. While some fluctuation in the percentage germination was apparent, the differences were not excessive and probably resulted from variations in greenhouse conditions. The microflora of the second soybean sample, held at 13.3 per cent moisture content, did not change greatly during the first 25 days, but after 34 days bacterial count was high. Germination of this sample decreased during the storage period, varying from 64 per cent after 6 days' storage to 29 per cent after 34 days. In contrast, the high moisture sample changed materially during storage in the number of infected seeds

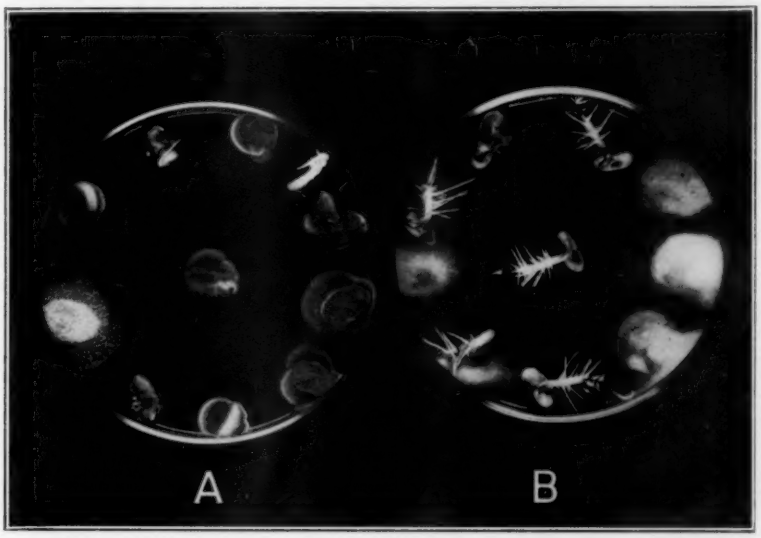

FIG. 2. Microflora of soybean seed. A. High-moisture-content seed with *Aspergillus spp.* predominant. B. Low-moisture-content seed with *Alternaria sp.* predominant.

present and in the marked decrease in viability of the seeds. The number of non-infected seeds decreased from 64 per cent after 6 days' storage to 1 per cent after 34 days' storage. Species of *Aspergillus* and *Penicillium* were obtained most frequently from infected seeds, about two-thirds of such seeds producing *Aspergillus spp.*, principally *A. glaucus, A. ochraceus* and *A. flavus,* while one-third of these seeds were infected with *Penicillium sp.* (Fig. 2). Germination dropped rapidly, from 46 per cent after 6 days' storage to 2 per cent after 34 days. Seed treatment increased the stand (Table 4).

FUNGI IN RELATION TO LOSS IN VIABILITY OF SOYBEAN SEED

Many fungi were obtained in the microflora studies; and a survey of their effects on the viability of soybeans was made. Isolates of *Aspergillus flavus, A. ochraceus, A. glaucus* (5 distinct isolates), *A. fumigatus,* and 3 unidentified species of *Aspergillus, Penicillium sp., Alternaria sp., Chaetomium*

TABLE 4.—*Changes in microflora and viability of Minsoy soybean seeds resulting from storage of seeds at different moisture contents*

Microflora, seed viability, and seedling vigor	Moisture content of beans and number days in storage											
	7.9 per cent				13.3 per cent				20.4 per cent			
	6	13	25	34	6	13	25	34	6	13	25	34
Microflora	Percentages of seeds infected with microorganisms											
Sterile	68	72	77	71	79	66	67	44	64	16	7	1
Alternaria	22	18	16	14	10	16	20	14	23	12	17	25
Aspergillus and *Penicillium*	0	0	0	1	1	6	1	1	2	65	74	68
Bacteria	4	5	7	14	6	4	12	41	5	0	0	6
Misc. fungi	6	5	0	0	4	9	0	0	6	8	2	0
Seed viability	Percentages of seeds germinating											
No treatment	73	60	67	53	64	54	46	29	46	18	13	2
Arasan-treated seed	74	58	76	70	61	59	66	37	57	50	20	5
Seedling vigor	Percentages of plants with first leaves expanded											
No treatment	93	93	88	89	95	83	63	84	87	72	23	0
Arasan-treated seed	95	86	92	95	90	88	83	85	93	86	25	10

sp., Cephalothecium roseum, Cunninghamella echinulata, and *Fusarium sp.,* were grown on potato-dextrose broth, 35 cc. of media in 100-cc. bottles, and on potato-dextrose agar in Petri dishes. After 10 days' incubation, the mycelial mats were removed from the broth cultures, and 15 cc. of the culture media placed in sterile Petri dishes containing 100 Illini soybean seeds.

TABLE 5.—*The influence of Aspergillus spores and staled culture media on the germination and vigor of Illini soybeans; notes taken 7 days after planting*

Fungus	Seeds soaked in staled culture media[a]				Seeds soaked in spore suspension[b]			
	24-hr. soak		48-hr. soak		24-hr. soak		48-hr. soak	
	Seeds germinating	Plants with 1st leaves expanded	Seeds germinating	Plants with 1st leaves expanded	Seeds germinating	Plants with 1st leaves expanded	Seeds germinating	Plants with 1st leaves expanded
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
A. flavus	15	0	0	0	36	0	2	0
A. ochraceus	26	42	37	5	78	86	64	84
A. glaucus No. 5	84	90	79	80	77	84	60	83
A. glaucus No. 10	86	87	74	77	78	85	67	91
A. fumigatus	82	82	85	84	77	84	72	82
A. niger	2	0	0	0	50	68	47	55
None (check)	74	85	74	88	72	83	69	88

[a] For the check, seeds were soaked in sterile potato-dextrose broth.
[b] For the check, seeds were soaked in sterile distilled water.

The seeds remained in the liquid for 24- and 48-hour periods, after which duplicate rows, 100 seeds to the row, were planted in sand in a greenhouse at 70° F. Petri-dish cultures were washed with 25 cc. sterile water and 15

12. Compositen: Bei *Serratula tinctoria* L. (Bot. Garten Jena und unbekannte Herkunft) zählte ich 2n = 22 Chromosomen (Abb. 2d). Für *Hypochaeris glabra* L. (Bot. Gärten Kopenhagen und München) wurde während meiner Untersuchungen von NEGODI (1936a, 1936b) die an Pflanzen aus Algier ermittelte Zahl n = 6 bekanntgemacht. In dem von mir studierten Material zweier Herkünfte fand ich jedoch nur 2n = 10 Chromosomen (Abb. 2e). Es ist zu erwarten, daß eine geplante Nachuntersuchung an Pflanzen aus Schleswig-Holstein auch diese Zahl ergeben wird.

trose broth	78	83	74	88	72	81	74	76
None	90	91	96	94	88	91	90	89

Only the culture media in which *Aspergillus flavus, A. ochraceus,* and *A. niger* had been grown affected seed germination appreciably, while of the spore suspensions only that from *A. flavus* affected the stand and vigor of the seedlings, although *A. niger* reduced the germination (Table 5). A severe stunting of seedlings resulted from treatment of the seed with the staled culture medium on which *A. flavus* had grown. Treatment of seed with a spore suspension of *A. flavus* also resulted in stunted seedlings. In addition to the reduced seedling vigor, poorer stands resulted from treatment of seed with spores of *A. flavus* and with the staled culture medium on which this fungus had grown. Thomas (10), working with filtrates of several fungi, obtained similar results on wheat. He found that strains of

A. flavus produced in culture media a toxic product that reduced the viability of wheat seed.

A more detailed test was made of the effects of *Aspergillus flavus* and *A. niger*. Cultures of the fungi were grown on potato-dextrose broth and agar, and after 5 days the inoculated potato-dextrose broth was passed through filter paper after removal of the mycelial mat. Seeds were soaked in the filtered culture medium and in a spore suspension of the fungi for 12 hours. Although the filtration did not remove all spores from the broth, the number of spores present in comparison to that in the spore suspension was very small. The seeds were then placed between filter paper to remove excess moisture. Other seeds were dusted with spores of the fungi, by

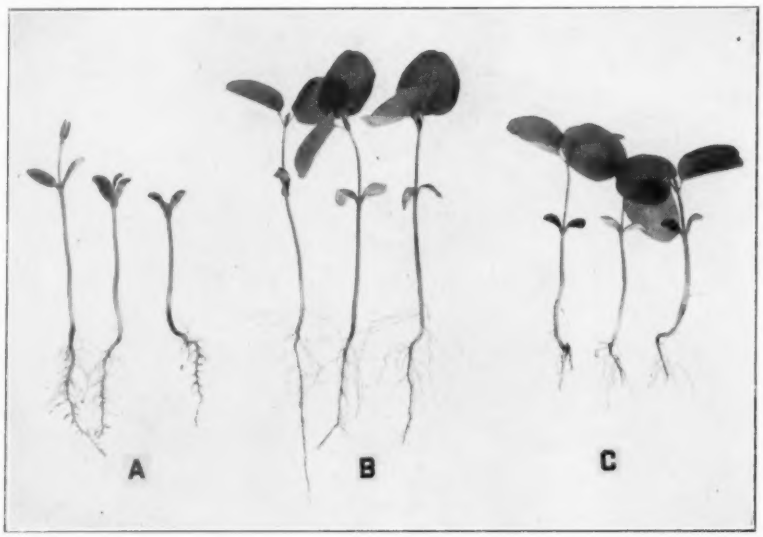

FIG. 3. Effect of *Aspergillus flavus* and *A. niger* on seedling development of soybeans. A. From seeds dusted with spores of *A. flavus*. The cotyledons are large and somewhat swollen while the plumule is greatly retarded in its growth. B. From non-inoculated seeds. C. From seeds soaked for 12 hours in liquid medium on which *A. niger* had grown. The seedlings are stunted but development of the plumule has not been retarded.

placing 100 seeds on a profusely sporulating culture in a Petri dish. For controls, seeds were soaked in sterile broth and in sterile water. Seeds from each of these lots, after having been dried on filter paper, were treated with the maximum-adhesion dose of Arasan; and duplicate 100-seed rows were planted in sand in a greenhouse at 70° F. immediately after treatment.

The results were similar to those obtained earlier with *Aspergillus flavus*, but the staled culture medium of *A. niger*, while reducing germination, did not give such pronounced results as in the previous experiment (Table 6). However, the fungi were grown for only 5 days in broth, and the seeds were soaked in the staled culture medium for 12 hours in contrast with the earlier soakings of 24 and 48 hours. Seeds dusted with spores of *A. flavus*

gave rise to seedlings that were abnormal. These plants were tall and the percentage germination excellent, but the production of the first leaves lagged behind those of the check, the plants resembling very closely the weak seedlings shown in figure 1. Moreover, there was a tendency for the cotyledons to remain full size, or even increase their size, while at the same time they retained their dark green color. Seedlings of the same age from seeds inoculated with dry spores of *A. flavus* and then treated with Arasan grew normally, a strong plumule being formed in two weeks, the cotyledons by then beginning to shrivel and turn yellow (Fig. 3).

Only 49 per cent of the seeds germinated after soaking for 12 hours in staled culture medium of *A. flavus*, and all of the seedlings were stunted. Treatment of the seed with Arasan increased germination slightly, but the seedlings were still dwarfed. Somewhat higher germination (66 per cent) occurred after the seeds were soaked in the spore suspension of *A. flavus* and 36 per cent of these seedlings were normal. Seed treatment increased the stand to 76 per cent, and 79 per cent of the seedlings were normal. It is evident also that soaking of seeds in sterile water, as Eyster (3) earlier showed with soybeans, or sterile broth for 12 hours prior to planting reduces the stand.

Further tests with *Aspergillus flavus, A. niger,* and *A. ochraceus* showed that the spores of *A. flavus* alone affected the vigor of soybean seedlings, while seeds covered with spores of *A. niger* and *A. ochraceus* produced normal seedlings.

DISCUSSION

The results show definitely that microorganisms, especially fungi belonging to the genus *Aspergillus,* are important in the loss of viability of soybeans in storage and probably are responsible for much of the heating of soybeans up to temperatures of 40–45° C. Seeds with a high moisture content, 13 per cent and up, become moldy when stored at room temperatures, and many of the seeds are infected with *Aspergillus spp.* High-moisture samples of soybeans, stored under conditions of increasing temperature, also have increasing numbers of seeds internally infected with *Aspergillus spp.* The number of infected seeds reached a maximum at about 45° C. and diminished at higher temperatures, until at 60° C. no seeds were found infected by fungi or bacteria. The close association of changes in the composition of the seed flora with rising temperature indicates that fungi, especially *Aspergillus spp.* are responsible for much of the heating. It is probable that the respiratory activities of these microorganisms contribute much to the high respiration rates observed by Ramstad and Geddes (8) in soybeans stored at moderately high temperatures.

Soaking seeds in filtered culture media on which species of fungi isolated from soybean seed had grown demonstrated the sharp specificity of 3 species of *Aspergillus* (*A. flavus, A. niger,* and *A. ochraceus*) in causing abnormal seedling development, while 11 other fungi, including *Alternaria* and *Fu-*

sarium spp., had no effect on seed germination. In addition, seeds dusted with spores of *A. flavus* gave rise to seedlings in which the plumule of many plants developed very slowly, producing retarded seedlings. However, spores of *A. niger* or *A. ochraceus* had no effect on seed germination or on seedling vigor. It is possible that spores of *A. flavus* developed on moldy soybeans in storage could be spread to uninjured seed, resulting in the development of weak seedlings from such seed. Fungicidal treatment of the seed dusted with spores of *A. flavus* counteracted the effect of the fungus spores, permitting normal seedlings to develop.

SUMMARY

A survey of the microflora of soybean seeds from Central and Southern Minnesota in 1942 showed that *Alternaria spp.* were most frequently found, with *Fusarium spp.* and bacteria next in frequency. The percentage of seeds infected with fungi and bacteria increased in proportion to the frost injury.

The percentage of seeds infected by fungi, especially *Aspergillus spp.*, increased as the moisture content of soybean seeds increased, while the viability and vigor of the high-moisture-content seeds, above about 13 per cent, rapidly decreased.

The percentage of seeds infected with *Aspergillus spp.* increased as the temperatures under which the seeds were stored increased. The maximum number of infected seeds was found at about 45° C., while at 60° C. no seeds were infected.

Aspergillus flavus, A. ochraceus, and *A. glaucus* predominated on infected seeds, while *A. niger* and *A. fumigatus* were less frequently found. *Cephalothecium roseum, Rhizopus nigricans, Cunninghamella echinulata, Chaetomium sp.*, and *Penicillium spp.* were also obtained from soybean seed.

Severe retardation in seedling growth resulted from storage conditions favoring development of *Aspergillus spp.* but fungicidal treatment of the seed improved the vigor and stand of the plants. Spores of *A. flavus* dusted on soybean seeds resulted in the retarded development of seedlings, while spores of *A. niger, A. ochraceus* or of any other fungus obtained from soybean seed caused no abnormal seedling development.

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RESISTANCE TO SEPTORIA LEAF SPOT AND ITS INHERITANCE IN TOMATOES[1]

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INTRODUCTION

The leaf spot caused by *Septoria lycopersici* Speg. has been one of the most injurious foliage diseases of tomatoes (*Lycopersicon esculentum* Mill.) particularly in eastern and central United States. Under circumstances most favorable for its development, it is capable of causing complete defoliation. An important degree of resistance to Septoria leaf spot, virtually unknown previous to 1941, has been discovered in a number of wild types of *Lycopersicon* by several independent observers (1, 4, 5, 6, 9), and breeding work has advanced to the point where control of the disease in commercial varieties seems definitely attainable. The present paper records the results of work at the Southeastern Regional Vegetable Breeding Laboratory on Septoria resistance and its inheritance. The work represents one part of an extensive program for the control of tomato diseases by plant breeding.

METHODS

The culture of *Septoria lycopersici* used in this work was isolated in 1941 from tomato leaves received from Dr. B. B. Higgins, Experiment, Georgia. The culture sporulates copiously on cooked bean pods. Figure 1 shows the semi-liquid masses of spores exuded from pycnidia on the bean pod.

Inoculum was prepared by macerating the sporulating cultures on cooked bean pods in an electric blender (2) for 2 minutes, then diluting with several volumes of water according to the strength of inoculum desired. Such inoculum contains, in addition to spores, large numbers of mycelial fragments on and imbedded in the cooked bean tissues. The strength of the inoculum had no effect on the character of the individual lesions; a very strong inoculum, however, produced multiple lesions whose coalescence caused early death and abscission of the leaves even on plants with the highest degree of resistance. Generally the desirable strength of inoculum for test purposes was that which produced lesions comparable in abundance to the infected leaves shown in figure 2.

Inoculation was accomplished by dipping the foliage of potted plants momentarily into a vessel of inoculum and then keeping them for 48 hours in glass-covered infection chambers in the greenhouse. The plants usually were 28 days from date of seeding, and ranged in height from 5 to 8 inches. Ordinarily a single series of inoculations involved about 1000 plants, which approximated the capacity of the infection chambers. In the inheritance

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study, samples of each parental type were included in each series of 1000 plants.

Leaf-spot readings were made on the seventh day after inoculation. Plants were graded both on the amount of injury and the character of the individual lesions developed upon them. The amount of injury varied with the temperature, strength of inoculum, and duration of time in the infection chamber; the characteristics of individual lesions, however, remained relatively constant. Later in the inheritance study, character of lesion was used as the sole basis of classification, and for this purpose only two types were recognized, *A* and *B*, as illustrated in figure 2.

FIG. 1. Culture of *Septoria lycopersici* on sterilized cooked bean pods. Left, sterile bean pod; Right, richly sporulating growth of *Septoria*. ×1.

Type *A* is the familiar Septoria leaf spot, evenly round, diameter 2 to 4 millimeters, with light-colored center usually containing from few to several black dots which are the pycnidia.

Type *B* lesion is definitely restricted, often pin-point in size and seldom more than 1 millimeter in diameter, dark reddish brown, and containing few or no pycnidia. Occasionally a leaf with type *B* lesions will also bear a few lesions up to 2 millimeters in diameter with light-colored centers, which are definitely intermediate between types *A* and *B*. An intermediate reaction of this type may suggest the behavior of a heterozygous genotype, but in the greenhouse studies the proportion of plants with the intermediate reaction was small and did not seem to correspond with any genotypic class.

In addition to the greenhouse inoculations, there was opportunity in 1942

and 1943 to observe plant reactions in the field following natural dissemination of *Septoria*. All of the inheritance data, however, are based on controlled inoculations in the greenhouse.

RESISTANCE TO SEPTORIA IN COMMERCIAL AND WILD TYPES OF LYCOPERSICON

Several attempts have been made by plant breeders to develop varieties with resistance to Septoria leaf spot and a few commercial types have been introduced which were claimed to possess some degree of resistance to the disease (3, 8). Variety tests in the greenhouse at this laboratory have failed

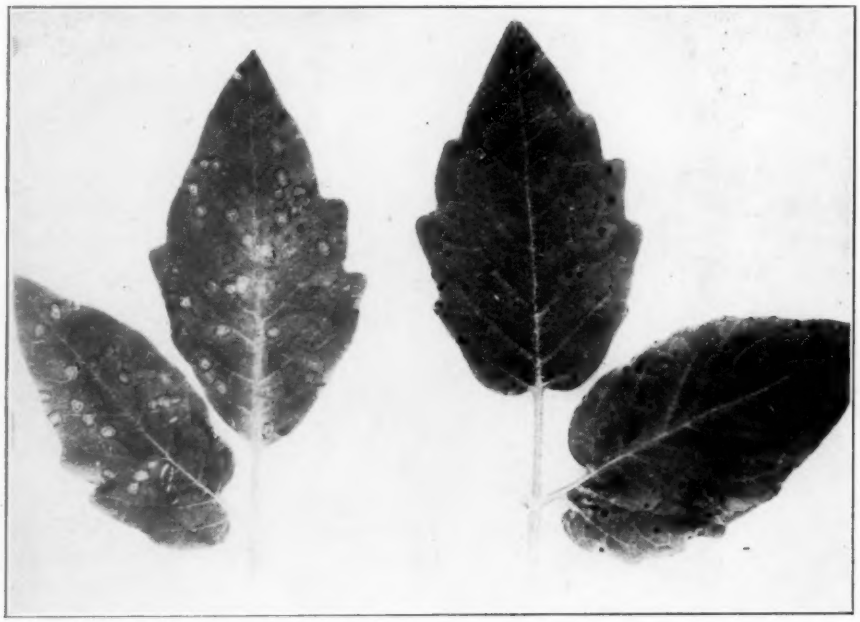

FIG. 2. *Septoria lycopersici* lesions on tomato leaves. Left, Type *A* lesions as they appear on susceptible commercial varieties; Right, Type *B* restricted lesions as they occur on resistant selection M6. ×1.

to demonstrate Septoria resistance in any commercial tomato. All of 127 commercial varieties tested by inoculation were highly susceptible.

In addition to the commercial varieties, this laboratory has tested by inoculation 267 accessions of *Lycopersicon* received through the Division of Plant Exploration and Introduction of this Bureau. None of these were immune to *Septoria*, but 12 possessed some degree of resistance, usually represented by rather small lesions intermediate between types *A* and *B*. None of the 12 were of the commercial *L. esculentum* type; 10 of them on the basis of fruit and foliage characteristics were judged to have resulted from outcrossing of *L. esculentum* with primitive types, either the currant tomato or the cherry tomato; and 2 were known to be of *L. hirsutum* ancestry. Certain other accessions of *L. hirsutum* Humb. and Bonpl. and *L. peruvianum*

(L.) Mill. were observed in the field to possess an important degree of resistance to *Septoria*. Our greenhouse observations on the resistance in wild types conform also to the field observations of Alexander (1); hence it is likely that any tomato resistant in the greenhouse will retain all or much of its resistance in the field.

Alexander (1) recorded evidence of resistance to Septoria leaf spot among 488 tomato introductions from the Division of Plant Exploration and Introduction. His data indicate that 67 of the 488 lines possessed some degree of resistance, but only 4 of the 67 approached the commercial types of *Lycopersicon esculentum* in fruit characters. The majority of the resistant lines belonged to the generally uncultivated species, *L. peruvianum, L. glandulosum* C. H. Mull., *L. hirsutum, L. pimpinellifolium* (Jusl.) Mill., and some not easily identified types possibly representing interspecific crosses. He found the highest degree of resistance in *L. hirsutum*.

Locke (6) also found some lines of *Lycopersicon hirsutum* to be highly resistant to *Septoria*, and the resistance appeared to be dominant in crosses with *L. esculentum*. Several *L. esculentum* × *hirsutum* hybrids supplied by Dr. R. E. Lincoln of the Indiana Agricultural Experiment Station have been inoculated at the Vegetable Breeding Laboratory and found to possess a very significant degree of resistance to *Septoria*. These lines, however, tend to be semi-sterile and have not been used in further breeding work.

The source of Septoria resistance used in heritance studies at the Vegetable Breeding Laboratory was a single plant selection derived from segregating lines of the Australian variety Targinnie Red. This variety apparently had become admixed with natural out-crosses so that many segregants were definitely non-*esculentum* in type. The Septoria-resistant segregant, called M6, was roughly intermediate between a currant and a cherry tomato in type of fruit and foliage. It proved to be completely fertile in crosses with commercial tomatoes. The Septoria resistance in M6 is equal in degree and apparently identical in type with that found in the best lines of *Lycopersicon hirsutum*.

The original Septoria-resistant segregant proved to be segregating in respect to resistance to Fusarium wilt (*Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder & Hansen), collar rot (*Alternaria solani* (Ell. and G. Martin) Jones and Grout), leaf mold (*Cladosporium fulvum* Cke.), and gray leaf spot (*Stemphylium solani* Weber) in addition to Septoria leaf spot. By continued selections certain lines were isolated which possessed a high degree of resistance to all five of these important diseases.

Breeding work with the Septoria-resistant tomato has advanced several generations. Original crosses were made in 1942 with the varieties Rutgers and Victor. True backcrosses were made to the original susceptible parents, and in addition the F_1 progenies were out-crossed with the varieties Marglobe, Indiana Marglobe, Gulf State Market, Montgomery, Stokesdale, and Pan America. Much breeding work remains to be done, yet the attainment of an important degree of Septoria resistance in commercial varieties seems probable.

INHERITANCE OF RESISTANCE TO SEPTORIA, IN FIRST, SECOND, AND THIRD FILIAL GENERATIONS

First generation hybrids from resistant × susceptible crosses were represented by four F_1 plants, all of which were resistant to *Septoria*.

Second generation hybrids were represented by 10 populations from crosses of M6 (the Septoria-resistant tomato) with the susceptible varieties, Rutgers and Victor (Table 1). Deviations from a 3:1 ratio were significant in 2 samples, which is slightly greater than expectancy. The combined F_2 ratio (709R to 207S) conforms very well with a calculated 3:1 ratio. By elimination of one sample of 29 plants the heterogeneity chi-square is low enough that the remaining populations can be combined satisfactorily. The 15 resistant plants in this separate sample are being grown for further study.

TABLE 1.—*Inheritance of Septoria resistance in ten F_2 populations from resistant × susceptible tomato crosses*

Cross	No. of plants			χ^2 (3:1)
	Total	Resistant	Susceptible	
M6 × Rutgers	122	98	24	1.847
	256	205	51	3.521
M6 × Victor	29	15	14	8.379**
	61	46	15	0.005
	81	57	24	0.926
	22	17	5	0.061
	23	19	4	0.709
	44	30	14	1.091
M6–13 × Victor	179	137	42	0.225
	99	85	14	6.226*
				21.990
	916	709	207	2.818
	Heterogeneity χ^2 (9 D.F.) = 19.172*			

* Significant at 5 per cent level.
** Significant at 1 per cent level.

Data for the third generation are confined to 26 families representing progeny of 26 plants classified "resistant" in F_2. According to the single factor hypothesis the F_2 resistant plants should prove to be either segregating or homozygous-resistant in the proportion of 2:1. This expectation was approximated, since of the 26 families, 18 were segregating (heterozygous) and 8 were resistant (homozygous).

The 3:1 type of inheritance is further indicated by the combined ratio within the 18 segregating F_3 populations, none of which deviated significantly from the 3:1 ratio (Table 2).

Classification of the plants was extremely simple; they fell into one of two reaction classes—either with the restricted type of lesion characteristic of the resistant parent (Fig. 2, right) or with the much larger light-centered lesion characteristic of most susceptible varieties (Fig. 2, left). No intermediate grades of plant reaction were distinguishable and dominance of the

TABLE 2.—*Inheritance of Septoria resistance in 18 segregating F_2 tomato families*

Cross	No. of plants			χ^2 (3:1)
	Total	Resistant	Susceptible	
M6 × Rutgers	19	17	2	2.123
	19	13	6	0.439
	18	11	7	1.852
	13	9	4	0.231
	18	14	4	0.074
	16	12	4	0.000
	17	10	7	2.373
	16	13	3	0.333
	15	8	7	3.756
	12	9	3	0.000
	16	12	4	0.000
M6 × Victor	19	11	8	2.965
	18	14	4	0.074
	15	10	5	0.556
	19	15	4	0.158
	18	13	5	0.074
	17	13	4	0.020
	12	10	2	0.444
				15.469
	307	224	83	0.678
	Heterogeneity χ^2 (17 D.F.) = 14.791			

factor for resistance seemed to be complete. Individual lesions of an intermediate type occurred infrequently on resistant plants, but the reaction of the plant as a whole was clearly either resistant or susceptible.

INHERITANCE OF RESISTANCE TO SEPTORIA IN BACKCROSSES TO THE SUSCEPTIBLE GENOTYPE

According to the single factor hypothesis, with resistance dominant over susceptibility, backcrosses to the susceptible parent should give progenies

TABLE 3.—*Inheritance of resistance to Septoria in backcrosses to susceptible tomato varieties*

Cross	No. of populations			χ^2 (1:1)
	Total	Segregating	Homozygous susceptible	
A. True backcrosses:				
(M6 × Rutgers) × Rutgers	10	5	5	0.000
(M6 × Victor) × Victor	10	2	8	3.600
B. Pseudo-backcrosses:[a]				
(M6 × Rutgers) × Gulf State	8	4	4	0.000
" × Montgomery	15	9	6	0.600
" × Pan America	16	7	9	1.000
(M6 × Victor) × Indiana Marglobe	6	3	3	0.000
" × Pan America	7	3	4	0.143
				5.343
	72	33	39	0.500
	Heterogeneity χ^2 (6 D.F.) = 4.843			

[a] To a genotype identical with an original parent with respect to factors under study.

one-half of which should be susceptible and one-half segregating (heterozygous). This expectation was nearly realized with 20 true backcross populations and 52 pseudo-backcross[3] populations (Table 3). None of the deviations from a 1:1 ratio are significant. The backcross of the F_1 to the resis-

TABLE 4.—*Inheritance of Septoria resistance in 33 segregating backcross tomato populations*

Cross	No. of plants			χ^2 (3:1)
	Total	Resistant	Susceptible	
(M6 × Rutgers) × Rutgers	70	52	18	0.019
	(18)[a]	(8)	(10)	
	54	32	22	7.136**
	53	39	14	0.057
	36	29	7	0.593
(M6 × Victor) × Victor	17	11	6	0.961
	19	14	5	0.018
Gulf State × (M6 × Rutgers)	52	42	10	0.923
	52	40	12	0.103
	(49)[a]	(15)	(34)	
	53	43	10	1.063
Montgomery × (M6 × Rutgers)	61	43	18	0.661
	82	66	16	1.317
	36	26	10	0.148
	20	17	3	1.067
	80	58	22	0.267
	88	59	29	2.970
	54	38	16	0.617
	54	42	12	0.222
	95	72	23	0.032
(M6 × Rutgers) × Pan America	81	57	24	0.926
	75	55	20	0.111
	82	61	21	0.016
	74	55	19	0.018
	(55)[a]	(14)	(41)	
	60	41	19	1.422
	79	57	22	0.342
Indiana Marglobe × (M6 × Victor)	19	17	2	2.123
	18	12	6	0.667
	19	14	5	0.018
(M6 × Victor) × Pan America	18	13	5	0.074
	36	29	7	0.593
	36	24	12	1.333
				25.817
	1573	1158	415	1.604
		Heterogeneity χ^2 (29 D.F.) = 24.213		

[a] Omitted from the final computation.
** Significant at the 1 per cent level.

tant parent offered no advantage from a horticultural standpoint, hence such crosses were not made.

Of the 33 segregating backcross populations, 3 deviated excessively from a 3:1 ratio (Table 4). The segregation in 2 of these populations in fact approximated a 1:3 ratio. The suggestion of reversal of dominance, how-

[3] The F_1 crossed to a genotype identical with an original parent with respect to factors under study.

ever, is hardly to be considered in view of evidence for a 3 : 1 type of inheritance supplied by the great majority of the data in Table 4 and elsewhere. It is believed that the 3 exceptional populations either represent true but exceptional deviations from a 3 : 1 ratio or are the result of error in handling seed stocks. Differentiation of the two reaction types was again very sharply defined and no misclassification is involved. Resistant plants from the deviating populations were saved for further study.

DISCUSSION

A study of plant disease resistance based on observations made in the greenhouse usually raises the question of the extent to which indoor reactions conform to those that will occur in the field. Generally the agreement between field and greenhouse observations in vegetable plant disease studies has been very close. A few exceptions, however, have been reported and the present instance of resistance to Septoria leaf spot of tomatoes may be another. Locke (6) and the present writers, on the basis of indoor studies in each case, find Septoria resistance to be dominant in inheritance. Wright and Lincoln (9) and Lincoln (5) on the basis of field observations, found such resistance to be recessive. In the latter investigations, however, the authors considered there was some question as to the validity of their hybrid material which involved a cross of *L. peruvianum* var. *humifusum* (C. H. Mull.) with *L. esculentum*.

The apparent disagreement in respect to dominance of Septoria resistance may suggest lack of conformity between indoor and outdoor observations or it may suggest that different genetic factors are involved in the several crosses. On the other hand, neither of these apparent conclusions may be warranted. Our own observations, both in the field and greenhouse, reveal a close general agreement in Septoria reaction. Selected lines that produced the restricted type of lesion indoors produced them likewise in the field, but in addition the older leaves near the base of the stem bore somewhat larger lesions in the field, often with a few pycnidia. This reaction of older leaves tends to escape observation in greenhouse tests where young plants are used exclusively. In the field the presence of any lesions with pycnidia may lead one to classify the plant as fully susceptible, especially since the restricted lesions which distinguish the resistant plant may be obscured by other pathological conditions usually present on field-grown plants.

Considering the nature of the disease and the method of dissemination of the causal organism, it is probable that an intermediate degree of resistance, such as is revealed in these studies, will be greatly increased in value when plants possessing it are grown in solid stands more or less isolated from fully susceptible plants. The near-by susceptible plants are a prolific source of spores which produce many restricted lesions on the resistant plants; coalescence of many such lesions causes a degree of injury that would not occur where the source of reinfection is limited to plants of the resistant type. Thus the circumstances surrounding a field study of disease resistance are apt to result in an unfair evaluation of the resistance potentialities of

a plant, and for this reason alone one should not overlook the value of indoor studies where many factors are under at least some measure of control.

Regardless of the ultimate value of the degree of resistance to *Septoria* described herein, the restricted lesion characteristic is highly distinct and so far as is known it represents the highest degree of resistance to *Septoria* available in the genus *Lycopersicon*. This restricted lesion character is inherited as a single factor and is clearly dominant over full susceptibility. The symbols *Se se* (*Septoria*) are suggested to distinguish them from the symbols *S s* for simple inflorescence and *Sp sp* for self-topping as listed by MacArthur (7). Little is known concerning the linkages of the factor *Se se*, but parallel studies now under way indicate that it is independent of the factors for resistance to wilt (*Fusarium oxysporum* f. *lycopersici*) and to gray leaf spot (*Stemphylium solani*).

SUMMARY

All of 127 commercial varieties of tomatoes tested by inoculation were highly susceptible to leaf spot caused by *Septoria lycopersici*. Some degree of resistance to *Septoria* was found in 12 of 267 accessions of foreign origin. These 12 were known to be of *Lycopersicon hirsutum* or *L. peruvianum* ancestry, or their characteristics definitely indicated outcrossing with some wild type.

The highest degree of resistance is characterized by a restricted type of lesion much smaller than normal and bearing few or no pycnidia.

Inheritance studies of crosses of commercial varieties with Septoria-resistant lines were made on 916 F_2 and on 3630 F_3 and backcross plants involving 70 populations.

Septoria resistance as represented by the restricted type of lesion (symbol *Se se*) is shown to be inherited as a dominant single factor.

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INHERITANCE OF RESISTANCE TO THE COLLAR-ROT PHASE OF ALTERNARIA SOLANI ON TOMATO[1]

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INTRODUCTION

In an earlier paper (3) it was shown that resistance to the collar-rot phase of *Alternaria solani* (Ell. and Mart.) Jones and Grout, was present in several species, varieties, and selected lines of tomato (*Lycopersicon* spp.). It was also suggested that the control of this disease might be effectively approached through plant breeding. In the process of breeding a commercially desirable, resistant variety, several crosses were made between lines which were either clearly resistant or clearly susceptible to collar rot. Second generation seedlings from these crosses showed segregation for resistance. These facts led to a study of the inheritance of this resistance, both as an aid in determining the most appropriate crossing procedures and as an addition to the knowledge of hereditary factors in the tomato. This paper records the results of the inheritance study.

METHODS

Tomato plants to be used as parents were grown in a screened greenhouse where all original crosses were made. Three types of crosses were made: resistant × susceptible, resistant × resistant, and susceptible × susceptible. In the first type, two sets of reciprocal crosses were analyzed. The F_1 was crossed where possible to one or both parents or to parents apparently identical with the original lines in collar-rot reaction. Second and third generation plants were for the most part field-grown. Occasional individual hybrids from the latter were "off type" for their respective populations, indicating uncontrolled outcrossing in the field, and were discarded for the genetic study.

The classification of all parent lines and hybrids was made on seedlings grown and inoculated as described previously (3), and the same strain of *Alternaria* used in the earlier work was used here. The varieties Devon Surprize and Bonny Best also were inoculated with each series of plants tested, as resistant and susceptible checks respectively. In addition, uninoculated seedlings of Bonny Best were included as a check for the presence of interfering organisms possibly present in the soil of the inoculation bench.

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Previous experience in grading collar-rot severity had shown that a wide range of reactions might be expected in segregating lines. The five arbitrary infection classes (Fig. 1) established, the description of each, and the numerical index assigned, were as follows:

Infection class	*Description*	*Numerical index*
I	Dead	0
II	Bent: seedling alive but broken over at collar lesion	25
III	Intermediate: erect but with well-developed collar lesion	50
IV	Healed: with shallow or definitely healed lesion	75
V	Free: no collar lesion, or only minute points of infection	100

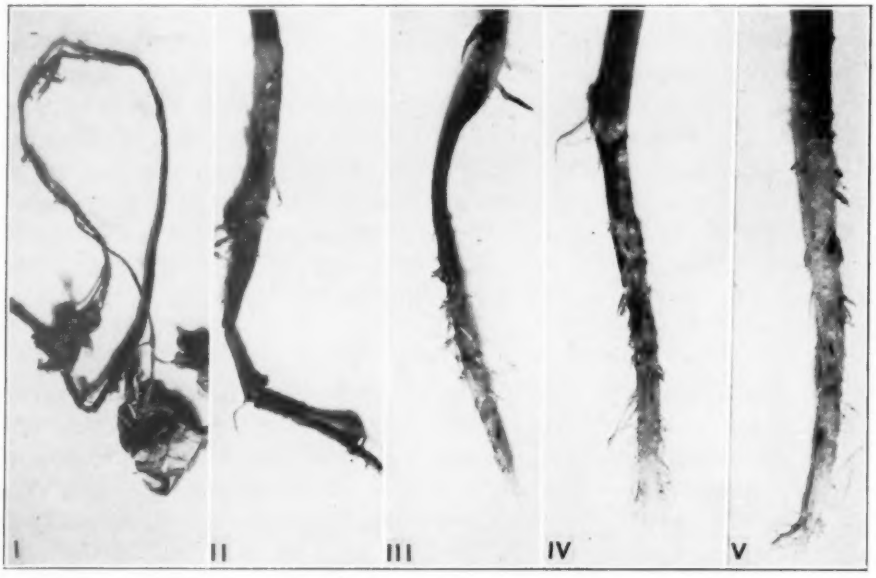

FIG. 1. Tomato seedlings illustrating the five arbitrary classes of collar-rot reaction: I, Dead; II, Bent; III, Intermediate; IV, Healed; and V, Free.

To get the collar-rot rating for any given line, the number of seedlings falling in each class was multiplied by the respective index of that class and the sum of the five products divided by the total number of seedlings inoculated. Thus low ratings indicated collar-rot susceptibility and high ones, collar-rot resistance.

GENERAL RESULTS

Collar rot developed abundantly in each series of inoculations. Devon Surprize maintained its resistance and Bonny Best its susceptibility. There was some difference in the severity of the disease developing in the different series, but the results of the inoculations were taken as recorded without any adjustment of the original method of classification.

In order to establish the grouping of the five infection classes most likely to represent genetic groupings, two sets of inoculations were analyzed. The first included F_1 seedlings and parents from ten crosses of resistant × sus-

ceptible lines. The results are in table 1. The mean rating of the nine different susceptible parents was 16.4 and of the resistant parents, represented by ten samples from Devon Surprize, was 86.1. The range of ratings observed, from 4.3 to 34.0 in the former and from 79.8 to 98.4 in the latter, illustrates the variations in reaction usually found in separate tests but also shows the sharp differentiation of susceptible and resistant lines. The F_1 ratings were higher than those of the respective susceptible parents in each case and the mean F_1 rating, 37.0, was clearly intermediate between the two mean parental ratings. In crosses 1 and 10, the F_1 ratings were not significantly higher than the ratings of the respective susceptible parents, but

TABLE 1.—*Collar-rot reaction of parents and of F_1 progenies in ten crosses, resistant × susceptible*

Cross No.	Parentage[a]	Collar-rot ratings			L.S.D.[d]
		Res. parent	Susc. parents	F_1	
1	Devon Surprize × P.I. 126,452[b]	81.8	22.7	28.2	7.9
2	Devon Surprize × F_4 (V.B.L. 245[c] × Marglobe)	79.8	6.9	18.8	8.4
3	Devon Surprize × Montgomery	88.7	16.9	35.6	8.5
4	Devon Surprize × Marglobe	82.2	17.3	50.0	9.2
5	Marglobe × Devon Surprize (reciprocal of cross No. 4)	98.4	18.4	42.1	11.9
6	F_4 (V.B.L. 245 × Marglobe) × Devon Surprize	86.0	8.0	52.3	19.1
7	F_4 (V.B.L. 245 × Marglobe) × Devon Surprize	93.8	34.0	53.6	10.2
8	Red Cherry × Devon Surprize	80.2	4.3	25.0	15.9
9	Red Pear A × Devon Surprize	89.2	17.7	31.4	10.3
10	Red Pear B × Devon Surprise	81.1	18.1	32.9	17.9
	Mean of ten ratings and its standard error	86.1 ± 2.0	16.4 ± 2.7	37.0 ± 3.8	10.5

[a] In these and other crosses, the female parent is listed first.

[b] P.I. numbers refer to introductions by the Division of Plant Exploration and Introduction of the Bureau of Plant Industry, Soils, and Agricultural Engineering.

[c] Regional Vegetable Breeding Laboratory selection (Red Currant tomato × Marglobe).

[d] Least significant difference at 5 per cent point between ratings of respective susceptible parents and F_1.

later experience with these crosses did not indicate that they differed genetically from the other crosses.

The intermediate reaction of the seedlings in a heterozygous condition, approaching the reaction of collar-rot-susceptible parents, suggests that susceptibility to collar rot is incompletely dominant over resistance.

The second set of inoculations used in establishing the most probable genetic groupings of collar-rot infection classes involved second and third generation plants. In the spring of 1942, random samples of F_2 seedlings from five crosses of resistant × susceptible lines (Nos. 3, 4, 5, 8, and 10, Table 1) were inoculated. All degrees of resistance and susceptibility appeared. After being graded and separated into the five established classes,

representatives of the four living classes were transplanted to the field. A total of 371 of these plants lived and reached maturity. Seed was collected from individual plants and the F_3 seedlings inoculated. The results are in table 2.

Of the four collar-rot classes assigned to the F_2 seedlings, represented in the first column of table 2, classes IV and V were the most nearly alike in their proportions of resistant, segregating, and susceptible F_3 families produced. The mean ratings of the two groups of F_3 families, 73.5 and 76.0, did not differ significantly, and it was evident that the F_2 seedlings assigned to these two classes were largely resistant since four-fifths of them (165 in 208) had uniformly resistant progenies.

TABLE 2.—*Collar-rot reaction of F_3 families from inoculated and classified F_2 plants from eight crosses, resistant × susceptible*

F_2		F_3 families from classified F_2 plants			
		Collar-rot reaction			Collar-rot ratings of all families
		No. of families			
Collar-rot classification	No. of seedlings	Uniformly resistant	Segregating	Uniformly susceptible	Mean rating and standard error
II, Bent	25	0	14	11	25.5 ± 4.32
III, Intermediate	138	15	99	24	38.5 ± 2.11
IV, Healed	99	79	20	0	73.5 ± 2.14
V, Free	109	86	23	0	76.0 ± 6.95

In contrast to the similarity of classes IV and V, the reactions of F_3 families from seedlings classed II and III were significantly different. This is particularly true since all three types of reaction appeared from class III plants and only two from those in class II. The mean ratings of all families from the two groups, 38.5 and 25.5, respectively, also differed significantly. The sharper difference between the ratings of families from classes III and IV gives support to the establishment of a dividing line between these two arbitrarily assigned degrees of collar-rot severity.

The presence of segregating F_3 families from the 208 F_2 seedlings graded "healed" or "free" indicated that they were not all homozygous for collar-rot resistance, or, in other words, the grading system was not infallible for every individual seedling. For this reason genetic analysis based on collar rot segregation in the F_2 from crosses of resistant × susceptible lines would not be reliable. It was decided to inoculate both F_2 and F_3 families, but to rely on the latter for critical genetic analysis.

In the first set of inoculations, involving F_1 plants, seedlings of Devon Surprize appeared for the most part in classes IV and V, the two most resistant classes, but some also were found in class III. Repeated progeny tests of Devon Surprize seedlings in class III have invariably indicated resistance equal to that in progeny tests of seedlings put in classes IV and

V in the same inoculations. This has also been true of other collar-rot-resistant parent varieties. Susceptible parent lines, on the other hand, occasionally gave a small proportion of their seedlings in the resistant classes, IV and V. Progeny tests of these seedlings have indicated susceptibility, suggesting that during the inoculation, occasional seedlings have escaped infection.

For the purpose of the present study, lines that resembled Devon Surprize in collar-rot reaction were considered resistant. In these, the great majority of inoculated seedlings were assigned to classes IV and V with a minority in class III. Lines resembling any of a number of commercial varieties such as Marglobe, Rutgers, or Stokesdale in collar-rot reaction were considered susceptible. Inoculated seedlings of the latter varieties were put largely in classes I and II with some in class III and only very rarely were any in classes IV or V. Lines which consistently gave seedlings in at least four of the collar-rot classes were considered to be segregating.

RESISTANT × SUSCEPTIBLE CROSSES

Fifteen crosses were completed involving three collar-rot-resistant parents (Devon Surprize, Targinnie Red, and a selection from P.I. 127,833) and several collar-rot-susceptible parents. The results of the collar-rot inoculations of the F_2 seedlings from these crosses are in table 3.

TABLE 3.—*Collar-rot reaction of F_2 seedlings from 15 crosses, resistant × susceptible*

Cross No.	Parentage	No. inoculated seedlings			χ^2 3 : 1
		Total	Infection class		
			I, II, III	IV, V	
1	Devon Surprize × P.I. 126,452	263	208	55	2.343
2	Devon Surprize × F_1 (*L. pimpinellifolium* × Marglobe)	180	150	30	6.667*
3	Devon Surprize × Montgomery	174	130	44	0.031
4	Devon Surprize × Marglobe	695	539	156	2.418
5	Reciprocal of cross No. 4	465	350	115	0.018
6	Reciprocal of cross No. 2	148	119	29	2.306
7	F_2 (*L. pimpinellifolium* × Marglobe) × Devon Surprise	182	134	48	0.183
8	Red Cherry × Devon Surprize	478	356	122	0.070
9	Red Pear No. 415 × Devon Surprize	248	182	66	0.344
10	Red Pear No. 414 × Devon Surprize	460	335	125	1.159
11	Targinnie Red × Montgomery	337	241	96	2.185
12	Devon Surprize × Victor	51	36	15	0.529
13	Reciprocal of cross No. 12	46	37	9	0.725
14	Vetomold × Devon Surprize	131	105	26	1.855
15	Marglobe × P.I. 127,833	221	154	67	3.332
					24.165
		4,079	3,076	1,003	0.367
			Heterogeneity χ^2		23.798*

* Significant beyond the 5 per cent point.

In each cross seedlings appeared in at least four of the collar-rot classes, and in most crosses in all five classes, indicating unquestionable segregation for resistance. The numbers of seedlings classified "IV, V," were found to be roughly one-fourth of the total number inoculated. This is also indicated by the relatively low chi-square values obtained for a 3 : 1 ratio, only one exceeding the 3.841 required for a significant deviation at the 5 per cent point. The excess number of seedlings in this exceptional cross, No. 2, occurred in the more susceptible classes. This deviation in one out of 15 crosses is not considered unusual in view of the experience regarding occasional misclassification of individual seedlings. The heterogeneity chi-square value, barely significant at the 5 per cent point, was reduced to non-significance by omitting this cross, and the accumulated chi-square value of 0.367 indicated a very close fit to the assumed ratio.

From five of the crosses, F_2 populations were grown to maturity and seed saved from individual plants at random. The collar-rot reactions of 251 families from the five crosses are in table 4. A total of 5,496 seedlings was classified in determining the reaction of the 251 families. Variations were

TABLE 4.—*Collar-rot reaction of 251 F_3 families taken at random from five crosses, resistant × susceptible*

Cross No.	Parentage	No. of F_3 families				χ^2 1 : 2 : 1
		Total	Susceptible	Segregating	Resistant	
4	Devon Surprize × Marglobe	47	12	26	9	0.915
5	Marglobe × Devon Surprize	65	10	38	17	3.369
10	Red Pear × Devon Surprize	46	15	21	10	1.391
14	Vetomold × Devon Surprize	47	15	22	10	1.255
15	Marglobe × P.I. 127,833	46	6	27	13	3.478
						10.408
		251	58	134	59	1.159
					Heterogeneity χ^2	9.249

found in the proportions of the three classifications but none deviated significantly from a 1 : 2 : 1 ratio as was shown by the chi-square values, none of which exceeded 5.991. The non-significant heterogeneity chi-square value indicated that the segregation was essentially the same in each of the five crosses.

RESISTANT × RESISTANT CROSSES

Four crosses were made involving parents classified as collar-rot resistant. The results of the inoculations of these crosses in the first and second generations and of two of the crosses in the third generation are in section A of table 5. All seedlings in the first and second generations of all crosses were resistant. In crosses No. 41 and 43, a total of 78 F_3 families were uniformly resistant with the exception of one segregating family in cross No. 43. All ratings were over 50, the lowest being 59 for the F_1 in cross No. 42.

In this and related studies, segregating populations have occasionally appeared in lines thought to be uniformly resistant. In some instances this has been traced to field crossing. Although no supporting data are at hand, the presence of one segregating F_3 family in a resistant × resistant cross (cross No. 43, Table 5) is thought to be due to chance pollination of a resistant F_2 plant in the field by one of several collar-rot-susceptible plants growing in the field at the same time. The possibility of other factors affecting collar-rot resistance is not, of course, excluded.

SUSCEPTIBLE × SUSCEPTIBLE CROSSES

Seven crosses were made involving parents classified as collar-rot susceptible. The results of the inoculations of these are in section B of table 5. All parents and first and second generation populations were susceptible. In contrast to the reactions of the previous group of crosses, all ratings were below 50, the highest being 31 in the F_2 of cross No. 46. Some of the variation in the ratings of individual lines is due to the fact that several separate inoculation series are represented in the table. In all cases, however, the parents and hybrids of any one cross were included in a single inoculation series.

TABLE 5.—*Collar-rot reaction in four resistant × resistant crosses and in seven susceptible × susceptible crosses*

Cross No.	Collar-rot reactions and ratings (in italics)[a]			
	Parentage	F_1	F_2	F_3 families
	A. *Resistant × resistant crosses*			
41	P.I. 127,814 × Dev. Surprize	resistant	resistant	33 families resistant
	66 *82*	*81*	*83*	
42	Dev. Surprize × Red Cherry	resistant	resistant	
	88 *96*	*59*	*86*	
43	Dev. Surprize × P.I. 126,923	resistant	resistant	44 families resistant, 1 segregating
	88 *94*	*71*	*91*	
44	Norduke × Dev. Surprize	resistant	resistant	
	86 *99*	*85*	*91*	
	B. *Susceptible × susceptible crosses*			
45	P.I. 128,602 × P.I. 95,588	susceptible	susceptible	
	12 *5*	*20*	*27*	
46	P.I. 126,452 × Marglobe	susceptible	susceptible	
	26 *16*	*25*	*31*	
47	Marglobe × Montgomery	.....	susceptible	
	5 *3*		*7*	
48	Montgomery × Marglobe	susceptible	susceptible	
	3 *5*	*2*	*5*	
49	Red Pear × Cooper Special	susceptible	susceptible	
	3 *2*	*8*	*5*	
50	P.I. 118,324 × Montgomery	susceptible	susceptible	
	5 *3*	*17*	*4*	
51	P.I. 95,588 × Marglobe	susceptible	susceptible	
	10 *5*	*0*	*9*	

[a] Figures under variety names or progeny groups represent numerical reaction ratings.

In making the collar-rot classifications listed in table 5, 229 F_1, 1402 F_2, and 1838 F_3 seedlings were inoculated, in addition to approximately 400 seedlings from the parents of the crosses.

BACKCROSSES

Three backcrosses, (resistant × susceptible) × susceptible, were tested. In addition F_1 plants were outcrossed to other collar-rot-susceptible parents

TABLE 6.—*Collar-rot reaction in second generation populations of backcrosses. A, (resistant × susceptible) × susceptible; B, (resistant × susceptible) × resistant*

Cross No.	Parentage[a]	Number of second generation populations				χ^2 1:1
		Total	Susceptible	Segregating	Resistant	
	A. *F_1 × susceptible parent*					
16	Cross No. 12 × Rutgers	21	9	12	0	0.428
17	" " × Valiant	14	7	7	0	0.000
18	" No. 13 × Rutgers	19	11	8	0	0.474
19	" " × Valiant	15	8	7	0	0.667
20	" " × Victor	17	5	12	0	2.882
21	" No. 4 × Gulf State	18	6	12	0	2.000
22	" " × Marglobe	4	1	3	0	1.000
23	" " × Stokesdale	14	4	10	0	2.571
24	" " × Pan America	3	0	3	0	3.000
25	" " × Victor	13	7	6	0	0.769
26	" " × Redcap	14	6	8	0	0.286
27	" No. 5 × Rutgers	2	1	1	0	0.000
28	" " × Gulf State	3	2	1	0	0.333
29	" " × Stokesdale	13	4	9	0	1.923
30	" " × Pan America	4	2	2	0	0.000
31	" " × Mingold	11	2	9	0	4.454*
32	(Dobbies Champion × Victor) × Rutgers	9	5	4	0	0.909
33	" × Montgomery	2	0	2	0	2.000
34	" × Stokesdale	18	8	10	0	0.222
35	" × Pan America	24	14	10	0	0.667
36	" × Victor	16	8	8	0	0.000
						24.585
		254	110	144	0	0.455
						Heterogeneity $\chi^2 = 24.130$
	B. *F_1 × resistant parent*					
37	Cross No. 4 × Danish Export	35	0	16	19	0.257
38	" " × Devon Surprize	8	0	4	4	0.000
						0.257
		43	0	20	23	0.209
						Heterogeneity $\chi^2 = 0.048$

[a] Crosses 4, 5, 12, and 13 are listed in table 3.
* Significant beyond the 5 per cent point.

similar in reaction to the original parents, and these crosses are treated as backcrosses. Seed was saved from individual first generation plants of the backcrosses and second generation seedlings were inoculated. The results of the inoculations are in section A of table 6. The direct backcrosses are

Nos. 20, 22, and 36. A total of 4,802 seedlings was inoculated in classifying the 297 families.

On the assumption that a single pair of factors is involved in collar rot resistance as is suggested by the analysis of second and third generation populations, half of the populations from each backcross would be expected to be susceptible and half segregating. The chi-square determinations were made accordingly for a 1 : 1 ratio. As shown by the data in section A of table 6, in each of the 21 crosses only the two types of reaction occurred, none of the populations being uniform for resistance. One of the chi-square values exceeded 3.841, indicating a deviation from the assumed ratio significant at the 5 per cent point; but the chi-square value of the accumulated segregation, 0.455, showed that the ratio closely approached 1 : 1.

One backcross, No. 38 (Table 6, B), was made to the collar-rot-resistant parent, Devon Surprize. In addition, in cross No. 37, the F_1 was outcrossed to the variety, Danish Export. This variety had given collar-rot reactions as resistant as Devon Surprize. A 1 : 1 ratio of resistant to segregating populations was expected and a close approximation, 20 : 23, observed. None of the 43 populations was uniformly susceptible.

SEGREGATION WITHIN F_3 FAMILIES

As a further test of the suggested single factor hypothesis, analysis was made of the seedling segregation within 258 F_3 families. The families selected were those in which segregation was unquestionable, or those in which seedlings usually appeared in five of the collar-rot infection classes (Fig. 1). The results of the analysis, assembled from eleven separate inoculations, are in table 7. Each inoculation included families from one or more of the resistant × susceptible crosses listed in table 3.

In one of the eleven inoculations, No. 81, the chi-square value indicated segregation deviating from a 3 : 1 ratio; the other ten and the accumulated segregation closely approached this ratio. In the deviating inoculation, an excess of seedlings appeared in the more resistant classes, IV and V, suggesting that a relatively weak infection occurred. The tests of the F_3 seedlings as a whole, however, appear to confirm the suggested type of inheritance.

SEGREGATION WITHIN SECOND GENERATION FAMILIES OF BACKCROSSES

A summary was made of the seedling distribution in 102 clearly segregating second generation populations from (resistant × susceptible) × susceptible. There were 1,549 seedlings classified, and of these 1,182 were in classes I, II, and III and 367 in classes IV and V. This result gives a satisfactory fit to a 3 : 1 ratio, the chi-square value being 1.343.

DISCUSSION

It seems evident that resistance to the collar rot phase of *Alternaria solani* behaves as an inherited character and that a single pair of factors

controls this resistance. Several minor deviations represented by significantly high chi-square values for 3 : 1 or 1 : 2 : 1 ratios appeared in the observed segregations. In each case, however, the accumulated chi-square values have been low and in most cases heterogeneity chi-square values indicated similar reactions among a group of different crosses.

There was no indication that the resistance of Devon Surprize, which was used in the majority of crosses studied, differed in degree or type from that of other resistant parent lines or from homozygous resistant hybrids derived from crosses made. It is significant that this variety from Scotland, Targinnie Red from Australia, and P.I. 127,833 from Peru had apparently identical forms of resistance, both visually and from a genetic standpoint.

TABLE 7.—*Collar-rot classification in 258 segregating F_3 families from resistant × susceptible crosses, assembled from eleven inoculations*

Inoculation No.	No. of families	No. of inoculated seedlings			χ^2 3 : 1
		Total	Infection class		
			I, II, III	IV, V	
11	20	406	302	104	0.082
71	9	168	131	37	0.794
74	50	699	515	184	0.653
75	25	655	502	153	0.941
76	78	1,038	754	284	3.084
77	20	480	365	115	0.278
78	12	289	213	76	0.259
80	8	225	176	49	1.246
81	19	557	397	160	4.123*
82	5	134	109	25	2.876
83	12	299	215	84	1.526
					15.862
	258	4,950	3,679	1,271	0.363
					Heterogeneity $\chi^2 = 15.499$

* Significant beyond the 5 per cent point.

The great majority of inherited characters that have been studied in *Lycopersicon* have been reported to be controlled by single pairs of factors (9). Definite evidence of genetic resistance to disease in tomato has been found for such diseases as Fusarium wilt (4, 10), Stemphylium leaf spot (2), Septoria leaf spot (8), Cladosporium leaf mold (1, 5, 6, 11), and possibly western yellow blight (7). In the majority of these cases, resistance has been reported as almost completely dominant or completely recessive to susceptibility. In the present study seedlings heterozygous for collar-rot resistance were usually distinguishable from those homozygous for resistance or susceptibility and their collar-rot reactions more nearly resembled the reaction of susceptible lines. For these reasons, it is considered here that collar-rot susceptibility is only partially dominant over resistance.

In the case of *Cladosporium fulvum* Cke., Langford (6) found several types of resistance, assigned symbols to the factors involved, and suggested

the system of symbols employed as a model for subsequent reports defining resistance to other diseases in tomato. In his system, the initials of the Latin name of the disease were used to represent the genes for resistance and subscript letters represented the initial letters of the tomato variety in which resistance was first discovered. The factor for resistance to *C. fulvum* found in the variety Stirling Castle was given as "Cf_{sc}."

Although collar-rot resistance was found in several varieties at the same time (3), the variety Devon Surprize has been used most extensively in this study and will be designated as the typical resistant variety. The symbols suggested are "A_d–a_d," the factor pair governing resistance to the collar-rot phase of *Alternaria solani*, as in the tomato variety, Devon Surprize.

Linkage relationships have not as yet been determined, but one contributing fact was observed; Devon Surprize has even-ripening fruit, the genes for which are located on chromosome No. VII of MacArthur's (9) chromosome map of tomato. This character was inherited independently of collar-rot resistance, indicating that the genes for the latter are probably not located on chromosome VII.

SUMMARY

Tomato lines uniformly resistant and lines uniformly susceptible to the collar-rot phase of *Alternaria solani* were crossed and succeeding generations analyzed for resistance or susceptibility to the disease. Analysis was made of the collar-rot reaction of approximately 500 F_1, 5,400 F_2, 7,300 F_3 seedlings and, in addition, 4,800 seedlings from backcrosses. A total of 15 F_2 populations, 251 F_3 families, and 23 backcross populations involving crosses of resistant × susceptible parent lines was tested. Plants were artificially inoculated in the seedling stage, grown in soil in greenhouse benches and classified according to the severity of the development of collar rot.

Unquestioned segregation for resistance occurred in the hybrids and analysis of second and third generation and backcross populations indicated a simple form of inheritance involving one pair of factors. An intermediate type of reaction was observed in which susceptibility to collar rot appeared to be only partially dominant over resistance. Resistance manifested in plants of the second and later generations appeared to be equal to that found in parent lines. Resistance found in tomatoes from widely scattered sources appeared to be inherited in identical manner.

The forcing variety, Devon Surprize, was used as the principal collar-rot-resistant parent. Following a modification of the system of symbols described by Langford, the factor pair governing resistance to the collar-rot phase of *Alternaria solani* was assigned the symbols "A_d–a_d."

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MOSAIC, STREAK, AND YELLOWS OF CARNATION[1]

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INTRODUCTION

The literature dealing with viroses of carnation was recently reviewed by Creager[2] and the name "mosaic" was substituted for the name "yellows" for the disease originally described by Peltier.[3] Although Peltier[3] and Lamkey[4] did not definitely say that the disease was caused by a virus, they did demonstrate the infectious nature of the disease by grafting and showed that it was not caused by fungi or bacteria. Lamkey[4] concluded that yellows was infectious but not contagious. He also referred to peach viroses and tobacco mosaic and inferred that carnation yellows was a similar type of trouble. Based on priority of usage the name "yellows" should have been used by Creager[2] because his description and photographs of foliage symptoms show that he was working with the disease described by Peltier.[3] Lowered quality of flowers from yellows-affected plants was reported by Peltier and by Lamkey, but Creager associated the breaking of the color of flowers with this disease.

Viroses of carnation have been under observation in Washington since 1930 and actively investigated since 1937.[5] Early in the investigations it was observed that all commercial varieties of carnations were infected with viroses to a greater or lesser extent and that it was advisable to develop virus-free seedlings in order to study the disease. As the work progressed on virus-free seedlings, it became evident that 2 viruses were associated with the disease known as yellows. One of the viruses, the mosaic virus, was generally present in all commercial varieties. The other virus, the streak virus, varied greatly in prevalence in different greenhouses and varieties. Since commercial varieties are practically 100 per cent affected with the mosaic virus, the streak virus was not observed alone in commercial plantings but was always in combination with the mosaic virus. This combination of viruses is the cause of the disease called yellows. It appears that Creager has substituted the term "mosaic" for "yellows" without separating the two viruses. The leaves and bud listed as normal in figures 1 and 2 of Creager's article show mosaic symptoms, and those listed as affected with mosaic show symptoms of yellows. It is interesting that Lamkey stated that the terms "mottle leaf," "mosaic," or "yellow fleck and streak" might be more appropriate descriptive terms than "yellows."

[1] Published as scientific paper No. 612, College of Agriculture and Agricultural Experiment Stations, State College of Washington.

[2] Creager, D. B. Carnation mosaic. Phytopath. 33: 823–827. 1943.

[3] Peltier, Geo. L. Carnation yellows. Proc. Amer. Carnation Soc. 25: 29–35. 1916.

[4] Lamkey, E. M. R. A consideration of yellows. Proc. Amer. Carnation Soc. 26: 25–35. 1917.

[5] Wash. Agr. Exp. Stat. Ann. Repts. 48: 65–66. 1938; 49: 61–62. 1939; 50: 76–77. 1940; 51: 85. 1941; 52: 74. 1942; 53: 67. 1943.

SYMPTOMS

Mosaic

Slight mottling of the leaves with light-green irregular to elongate blotches (Fig. 1, C), which makes the plant a lighter green than normal, is the characteristic symptom of this disease. The mottling is usually more pronounced in the young leaves than in the older ones. Foliage from virus-free plants is smooth and uniformly green in contrast with the mottled foliage of mosaic-affected plants. Mosaic often shows in the flowers of colored varieties as somewhat lighter streaks that parallel the veins of the petals. The mottling of the foliage and the broken color of the flowers caused by mosaic ordinarily are not sufficiently damaging to be of much economic importance.

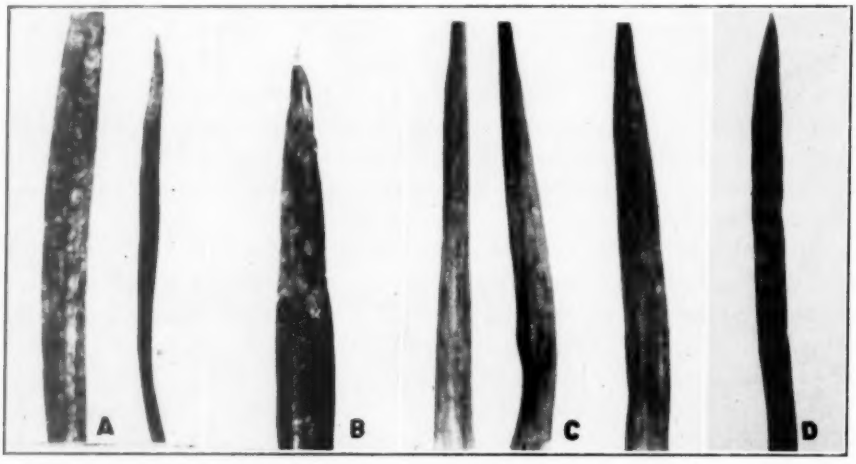

FIG. 1. Mottling and necrosis of carnation leaves caused by viruses. A, yellows; B, streak; C, mosaic; D, virus-free leaf.

Streak

Yellowish or reddish spots and streaks one-half to one mm. wide, either circular or elongate, paralleling the veins are noted on foliage of affected plants (Fig. 1, B). The reddish spots are more pronounced on foliage of colored varieties than the yellowish spots on foliage of light pink or white varieties. On the deep red varieties, such as Potentate, the outer portion of the spot becomes purple in contrast to the light tan center. Many of the lower leaves on affected plants may become severely spotted, turn yellow, and die. The streak virus alone appears to produce no distinguishing symptom on the flower.

Yellows

Since yellows is caused by a combination of the two viruses, mosaic and streak, both the mottling and spotting are characteristic of the trouble (Fig. 1, A). The severity of the foliage mottling and the flower streaking associ-

ated with the mosaic disease are increased by the presence of the streak virus. Severely spotted foliage more often dies with the combination of the 2 viruses than with streak alone. Yellows has been observed to cause the death of nearly all the foliage on flower stems of Virginia and Laddie varieties during March and April. Yellows-affected plants also suffer more severely from Fusarium branch and stem rot than is the case with mosaic-affected plants.

TRANSMISSION

Four series of grafts on 100 virus-free seedlings and 6 series of mechanical inoculations on 272 virus-free seedlings were made from yellows and mosaic plants in 1938–40 before determining that 2 viruses were associated with the yellows disease. Accordingly, the results of these tests were confusing. In October, 1940, grafts from yellows-affected Patrician and mosaic-affected King Cardinal were made on 5 plants, each of a virus-free seedling

TABLE 1.—*The transmission of the carnation mosaic and streak viruses*

Inoculum	Type of inoculation	Number of trials	No. plants inoculated	Average percentage of plants affected with		
				Mosaic	Streak	Yellows
Yellows	Mechanical[a]	6	316	99	0	0
Mosaic	"	2	20	100	0	0
Yellows	Grafting	6	86	.....	.....	90
Mosaic	"	3	20	100	0	0
Streak	"	1	12	0	100	0
Yellows	*Myzus persicae*	4	147	0	93	0
Yellows	*Thrips tabaci*	2	30	0	0	0
Mosaic	*Myzus persicae*	2	18	0	0	0
Yellows	" "	2	92[b]	.....	.....	94
Streak	" "	1	5[b]	.....	.....	100

[a] Portion of affected leaf rolled and injured on swab with a pot label before being rubbed on leaf to be inoculated. Leaf to be inoculated was sprinkled with carborundum dust previous to inoculation.

[b] Plants affected with mosaic before being inoculated in these tests.

clone. Symptoms of yellows developed on plants grafted with yellows-affected scions from Patrician, and mosaic on plants grafted with mosaic-affected scions from King Cardinal. This separation of symptoms was perpetuated in further grafts from affected plants to plants of another seedling clone.

Mechanical inoculations from yellows-affected plants previous to the understanding of the complex nature of the disease indicated that yellows was not transmitted mechanically, but that a mosaic-mottling of the foliage was readily transmitted by mechanical inoculation methods from yellows-affected plants. The use of aphids (*Myzus persicae* Sulzer) gave indications that yellows was transmitted by this insect, because the inoculated plants were carrying the mosaic virus before viruliferous aphids were placed upon them. An understanding of the situation was obtained when aphids were transferred from yellows-affected Patrician to 5 plants of a seedling clone

that had been previously inoculated with mosaic, and typical yellows developed on the dual-inoculated plants.

Since October, 1940, a large number of inoculations by mechanical methods and by the use of insects on virus-free clonal lines of seedlings has shown that the mosaic virus is readily transmitted by mechanical inoculation but is not transmitted by *Myzus persicae* or *Thrips tabaci* Lindeman (Table 1). Also, the inoculations have shown that the streak virus is transmitted by *M. persicae* but not by *T. tabaci* or by mechanical inoculation methods (Table 1). Preliminary tests show that the mosaic virus is inactivated *in*

TABLE 2.—*Observed reaction of carnation varieties infected with yellows in commercial greenhouses*[a]

Severe symptoms	Moderate symptoms	Slight symptoms
Betty Lou	Achievement	Admiration
Bonanza	Dark Scarlet	Carlotta
Boston Ward (3)	Del Ray	Dairy Maid
Camelia	Eleanor	Dictator
Chief Kokomo	Ethel	John Briry
Dimity	Ivory White	Matchless
Ditchling	Joan Marie	Mrs. Sims
Doris Allwood	Kathryn	Olivette
Fire Chief	Maytime	Pelargonium
Gardenia	Mrs. C. B. Johnson	Pharaoh
Giant Laddie	My Love	Pink Abundance
Golden Wonder	Paragon	Portland Pink
King Cardinal	Peter Fisher	Vivel Field Clark (2)
Martha Ellen	Pink Spectrum	Vivian (1, 2)
Melrose	Red Matchless (1)	
Patrician	Red Triumph (2, 3)	
Pink Treasure	Rosalie	
Potentate	Salmon Spectrum (2)	
Puritan (2)	Satellite (1, 2)	
Purity (3)	Spectrum Supreme	
Robert Allwood (2)	Topsy (3)	
Rose Pink Joan Marie	Virginia	
Scarlet Monarch (2)	Virginia Rose	
Senator		
Snow White		
Tangerine		
Triumph		
White My Love (2)		
Woburn (2)		

[a] Observations made in 1938–1944 in greenhouses in Washington and (1) Charles City, Iowa; (2) Milwaukee, Wisconsin; and (3) Denver, Colorado.

vitro near 60° C., and remains active *in vitro* for 7 days but is not active after 42 days. The minimum incubation period of the mosaic virus was 22 days and of the streak virus 25 days, but longer periods of incubation, up to 50 to 60 days, were commonly observed depending upon the vigor of the inoculated plants.

VARIETAL RESISTANCE

Carnation varieties in commercial greenhouses differ in severity of symptoms of yellows shown by affected plants. Observations in Washington, Iowa, Wisconsin, and Colorado recorded in table 2 show the degree of injury

noted on common varieties. Some of the varieties listed as slightly or moderately injured by the presence of yellows in the plant have not been observed under sufficiently varying conditions to be certain of their proper classification.

TABLE 3.—*Percentage of plants of carnation varieties showing symptoms of yellows in commercial greenhouses of Washington*[a]

Variety	Greenhouses	Range	Average
	No.	*Per cent*	*Per cent*
Pollyanna	1	0	.....
Matchless	3	0–7	2
John Briry	6	1–10	4
Dark Pink Maytime	1	10	.....
Eleanor	1	10	.....
Dorothy Napier	2	10–20	15
Hercules Virginia	2	10–20	15
Pink Treasure	1	20	.....
U. S. D. A. No. 1	1	20	.....
Olivette	4	0–40	25
Pharaoh	2	10–60	35
Maytime	3	2–70	41
Snow White	2	10–75	46
Peter Fisher	5	6–90	47
Joan Marie	7	20–80	54
Tom Knipe	3	40–70	50
Puritan	4	10–90	60
Pink Spectrum	4	30–90	61
Gardenia	1	67	.....
Virginia Rose	6	30–95	69
Pink Abundance	1	70	.....
My Love	5	60–100	78
Melrose	1	80	.....
Purity	1	80	.....
Rose Pink My Love	1	80	.....
King Cardinal	10	15–100	81
Chief Kokomo	2	75–90	83
Virginia	7	60–100	86
Camelia	1	90	.....
Hermosa	1	90	.....
Mrs. C. B. Johnson	1	90	.....
Ethel	2	90–98	94
Spectrum Supreme	4	90–100	94
Betty Lou	2	100	100
Fire Chief	2	100	100
Giant Laddie	1	100	.....
Paragon	1	100	.....
Patrician	2	100	100
Triumph	1	100	.....

[a] Observations made in greenhouses in March and April, 1941–1944. The highest percentage observed in a given variety is recorded.

Many of the varieties listed as showing severe symptoms have had sufficient loss of crop to lead to their discontinuance by some growers in Washington during the past 6 years. Most of the varieties listed in table 2 have been developed in the last 15 years. Matchless and Boston Ward appear to be the only varieties observed that have been in production for 30 years. This leads one to conjecture as to the part played by yellows in the "running-out" of commercial carnation varieties in the past. Peltier, in 1916,

stated that all varieties grown at that time had yellows and that the disease was the most serious trouble of carnations. Comments by a grower in 1916[6] that he had seen yellows as long as he had grown carnations would lead to the conclusion that it has been an important disease of carnations for a long time.

Yellows was prevalent on many carnation varieties in commercial greenhouses of Washington, in March and April of 1941–1944 (Table 3). The standard varieties Betty Lou, Camelia, Chief Kokomo, Gardenia, Giant Laddie, Melrose, Patrician, Puritan, and Purity have been discarded during

TABLE 4.—*Observations on yellows in rooted cuttings of carnations purchased by growers in Washington, 1940–1944*[a]

Source and variety of cuttings	Percentage of plants with symptoms of yellows
Massachusetts	
Bauerlein's White	0
Charm	0
Dark Pink Maytime	0
New York	
Olivette	0
Pink Spectrum	0
Rosalie	0
Snow White	0
Virginia Rose	0
Indiana	
Dorothy Napier	10, 20
Maytime	20
Olivette	2
Pharaoh	4
Pollyana	11
Tom Knipe	20
Vivian	10
Colorado	
Derigo	20
Light Pink Maytime	50
Pink Spectrum	58
Pink Spectrum Supreme	56
Spectrum Supreme	56
Oregon	
Melrose	50

[a] Data collected on young potted plants in greenhouses in Washington within 30 days after the plants had arrived from shipping points.

this 4-year period mainly because of reduced quality and production caused by the yellows disease. Some growers have also expressed the opinion that reduced quality and production makes it desirable to find new varieties to replace Fire Chief, Joan Marie, King Cardinal, My Love, Virginia, and Virginia Rose. High percentages of plants of these varieties were affected with yellows in the different greenhouses (Table 3). The John Briry and Matchless varieties consistently had none to low percentages of yellows. The fact that aphids are seldom found on these varieties except in heavily infested greenhouses no doubt accounts for their relative freedom from the disease.

[6] See footnote 3.

On the other hand, King Cardinal, Fire Chief, and some other varieties to a lesser extent are very susceptible to aphid infestation.

As the varieties become less productive, growers buy new stock from other parts of the country in order to obtain more productive old varieties or to try new varieties. Many lots of rooted cuttings brought into the State of Washington during the past 5 years have been affected with yellows (Table 4). No definite information was obtained as to the location of the greenhouse in which the cuttings were made but the state from which the cuttings were shipped is recorded in table 4. From these few data it appears that yellows is more prevalent in carnation stock produced in the central and western part of the United States than in stock produced in the northeastern part of the United States.

Seedling carnation plants were developed from seed obtained from England and France in 1938–1939, but crosses were made with commercial varieties in the greenhouse in Pullman in 1938–1940 for the production of seed. A total of 1038 seedlings have been grown with no indication that the mosaic or streak viruses were transmitted to the plants through the seed. Twenty-eight of the most promising seedlings were selected and developed into clonal lines. Inoculation by grafting on these clones has failed to show any immunity to infection by the streak and mosaic viruses, but considerable difference in degree of injury to affected plants has been observed. Two of the clones, that show relatively mild symptoms when affected with yellows have been distributed in lots of 100 and 1200 plants each to 7 growers in the Spokane area for observations under commercial conditions. The 2 varieties are dark rose pink and named Victoria and Genevieve. Victoria came from a cross of Joan Marie × King Cardinal and Genevieve from a cross of King Cardinal × an unnamed red seedling developed from seed purchased in England. Further crosses have been made but the war emergency has curtailed investigations on this project.

THE EFFECT OF NUTRITION ON SYMPTOMS

It was recognized by Peltier that symptoms of yellows were influenced by environmental conditions. Symptoms are often very marked on rooted cuttings in the sand or soon after they are potted. This is especially true if the cuttings remain in the sand beyond an optimal period for root development. The use of hormones to assist in rooting cuttings has been considered by growers to be responsible for the development of severe symptoms. Tests with hormones at Pullman indicate that symptoms are no more severe on treated cuttings than on untreated cuttings. There is the possibility that the more rapid rooting and greater abundance of roots on affected treated cuttings would make them suffer more severely from a depleted nutrient condition than untreated cuttings, if left in the sand too long before potting. Cuttings from yellows-affected plants do not strike root easily and lower percentages of rooted plants are obtained from them than from virus-free or mosaic-affected plants.

Observations and discussions with growers would lead to the conclusion that unfavorable growing conditions that injure the root system or check plant growth increase the development of symptoms in diseased plants. Low fertility of soil, excessive amounts of salts in the soil, too heavy and frequent applications of water, and excessive applications of fertilizer are factors that appear to have been responsible for severe symptom development in various greenhouses under observation. In the fall and winter the symptoms of yellows are usually mild unless aggravated by very unfavorable soil conditions. In the spring, however, when heavy growth is made with heavy watering and no fertilizing the symptoms may become very severe. Injury to the root system in potting from the sand, repotting, or transplanting has been observed as a possible factor in increasing symptoms.

Yellows-affected Patrician plants were placed in neutral peat in glass pots October 6, 1938, and grown through the winter with the addition of nutrient solutions as the moisture condition of the peat showed the need.

TABLE 5.—*The effect of nutrients upon the development of carnation yellows symptoms*[a]

Nutrient solutions	No. plants grown	Degree of symptoms on plants			Av. number spots per shoot
		Medium	Severe	Dead	
Complete nutrient	12	5	0	0	8
Complete minus nitrogen	12	3	6	3	47
Complete minus phosphorus	12	3	8	1	92
Complete minus potassium	12	4	8	0	74

[a] Patrician plants grown in pots of neutral peat.

Twelve pots each were treated with complete nutrient, complete nutrient minus phosphorus, and complete nutrient minus potassium solutions.[7] Severe symptoms appeared on the plants in the pots receiving complete nutrient minus phosphorus, and complete nutrient minus potassium solutions on October 18, 1938, but plants in the other solutions were free of severe symptoms. The final data taken February 6, 1939 (Table 5), show that the lack of nitrogen, phosphorus, or potassium from the complete nutrient solution gave increased symptoms on plants and that the plants were more severely damaged from a lack of potassium or phosphorus than from a lack of nitrogen.

The soil in 27 benches of carnations in 15 greenhouses was analyzed in March, 1942, according to the technique described by Spurway.[8] Tests were made for nitrates, potassium, phosphorus, chlorides, and soil reaction. Total salts in the soil of each bench were determined with the "Washington State Soil Tester" developed by E. C. McCulloch and L. C. Wheeting. The carnation plants in 12 of the benches were showing severe symptoms and in

[7] Acknowledgment is made to Dr. L. C. Wheeting for assistance in conducting the nutrient solution tests.

[8] Spurway, C. H. Soil testing—A practical system of soil diagnosis. Mich. Agr. Exp. Stat. Tech. Bull. 132 (Revised). 1935.

15 benches mild symptoms of yellows. No one condition or combination of conditions seemed to be a major factor in symptom development. Successive analyses of the soil throughout the growing season would be advisable if the relation of soil condition to symptom development is to be better understood.

CONTROL

Mosaic appears to be of little economic importance on carnations and since it is spread rapidly by mechanical contact in cultural practices there appears to be no practical method for its control. In the development of new varieties, care should be used to not handle the new seedling clones after handling commercial varieties.

Streak is transmitted readily by aphids, therefore the control of aphids is of the utmost importance in keeping this disease out of the plants. Because yellows is caused by transmission of the streak virus by aphids to plants already affected with mosaic, the control of aphids is very important if losses from yellows are to be avoided.

The mosaic and streak viruses are not transmitted in seed. Thus, new varieties developed from seed are free of mosaic, streak, and yellows. New varieties developed as sports from existing varieties are usually affected with the mosaic virus and often have yellows, depending upon the virus content of the mother plant. New varieties grown in the vicinity of commercial varieties, become affected with mosaic very rapidly when no care is used to prevent mechanical transmission of the virus in cultural practices. The rapidity of infection of new varieties with the streak virus depends upon the prevalence of this virus in adjacent stock and the transfer of aphids from diseased stock to plants of the new varieties.

Rogueing and selection of stock has not given complete control of yellows because the appearance of symptoms on diseased plants is not sufficiently distinct at any given time to allow for complete eradication. This was shown by an attempt to rogue yellows-affected stock for 2 growers in May, 1941. At benching time, stock showing no definite symptoms of yellows was selected from Joan Marie (2,065 plants) in one greenhouse and from King Cardinal (1,951 plants) in the other greenhouse. Observations on the plants in the resultant benches during the next season showed that a large number of infected plants escaped detection when the stock was rogued. Symptoms are usually more distinct as the cuttings come out of the sand or for a period of a month or 2 after removal from the sand. Careful selection of symptomless young plants from the time of removal from the sand until benching should be of great value in reducing losses from yellows. Symptoms on affected plants are usually so mild during the winter months that the selection of cuttings from only healthy plants appears impractical.

Growing carnations under optimal soil, fertility, and moisture conditions reduces losses from yellows. Unfavorable conditions that check the plant growth may increase symptoms and crop damage when the disease is present in the stock.

SUMMARY

Two viruses, mosaic and streak, are associated in the carnation disease known as yellows.

Mosaic shows as mottling of the leaves with light and dark green areas and slight color breaking in flowers of colored varieties with little damaging effect on the plant although generally present in all commercial varieties. Streak is characterized by yellowish to reddish spots and streaks on the foliage that may lead to death of portions of the plant. In commercial culture, streak is not observed alone but is in combination with mosaic to produce the disease known as yellows. The mottling of the foliage and breaking of color of the flowers caused by mosaic and the spotting of the foliage and death of portions of the plant caused by streak are intensified in the combination of the two viruses in the plant. Yellows has been very injurious to many varieties of carnations.

The mosaic virus is readily transmitted by mechanical inoculation but not by aphids or thrips, has a minimum period of incubation of 22 days, and is inactivated near 60° C. and after 42 days *in vitro*. The streak virus is transmitted by aphids but not by mechanical inoculation or by thrips, and it has a minimum period of incubation of 25 days.

Varieties of carnations differ in severity of damage caused by the yellows disease. The elimination of some varieties from commercial production, such as Betty Lou, Camelia, Chief Kokomo, Gardenia, Giant Laddie, Melrose, Patrician, Puritan, and Purity has been due largely to reduced production and lowered quality caused by yellows.

The 2 viruses were not transmitted in seed and considerable difference in susceptibility of seedlings was observed. Two virus-free and somewhat resistant seedling varieties are being tested in commercial greenhouses.

The development of symptoms of yellows is greatly influenced by environmental conditions. Unfavorable conditions that check plant growth, excess watering, low fertility, and excess salt in the soil, may enhance the development of symptoms.

The control of aphids is the most important practice to reduce losses from yellows. Careful selection of yellows-free planting stock is recommended, from the time the cuttings are removed from the sand until benching. The selection of yellows-free plants from which cuttings are removed does not appear to be practical since the symptoms of yellows are usually very mild during the winter months.

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MYROBALAN MOTTLE AND ASTEROID SPOT

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For the past five years two abnormalities, "mottle" and "asteroid spot," have been observed in myrobalan plum (*Prunus cerasifera*) seedlings used almost exclusively as root-stocks for propagating plums in fruit nurseries. Since World War II started in 1939, such seed comes almost exclusively from one nursery in California. Samples taken from shipments of seedlings from numerous nurseries as received in New York State have regularly disclosed the presence of these abnormalities during the growing season.

MOTTLE

Myrobalan mottle (Fig. 1) is characterized by an abnormal type of foliage development over the entire plant which is stunted. The leaves are small, irregular, undulating in surface, and contain dark green areas visible by reflected light.

Attempts to transmit mottle by budding or grafting from myrobalan plum to myrobalan plum, peach, cherry, and prune during 1941, 1942, and 1943 have been unsuccessful. On the other hand, the myrobalan mottle symptoms are regularly perpetuated on these plants in the growth from the affected buds. This tendency of bud perpetuation, but not of visible transmission, suggests a genetic phenomenon.

The origin of myrobalan mottle has been traced to the seed. Seed was obtained from the California nursery in 1942 and 1943 and grown in the greenhouse during the winter. Replicated plantings were made after variable periods of storage in moist peat at 5° C.[1] Each replicate consisted of 4 units of 40 or more seeds. Percentages of germination ranged from 90 to 100. It was discovered that at least one seedling had mottle symptoms in all replicated plantings of 120 or over of 1942 seed and of 50 or over of 1943 seed. Thus the percentage of seedlings affected with mottle ranged between 0.8 and 2.0 per cent in these two samples. These germination experiments leave no doubt that myrobalan mottle is seed-borne. When considered along with the fact that it is also bud perpetuated but not bud transmitted the conclusion is that myrobalan mottle is apparently a genetic abnormality originating at the seed source.

A variable percentage of myrobalan plum seedlings have been observed to contain the myrobalan mottle symptom inside and outside the State. In one case approximately 15 per cent of the seedlings showed this symptom in August although the percentage more commonly observed was between 0.5 and 5.0 per cent. No planting has been observed totally free from this abnormality.

[1] Usually a 3-month cold treatment was adequate for breaking dormancy of *P. cerasifera*.

ASTEROID SPOT

Asteroid spot or chlorotic spot describe a second abnormality on myrobalan plum, the first term describing the lesions on the foliage and the second those on the stems. At any time during the growing season few to many persistent star-shaped chlorotic spots (2 mm. ± in diameter) may appear on the leaves (Fig. 2, A), and cause distortion (Fig. 2, B). The chlorotic spots

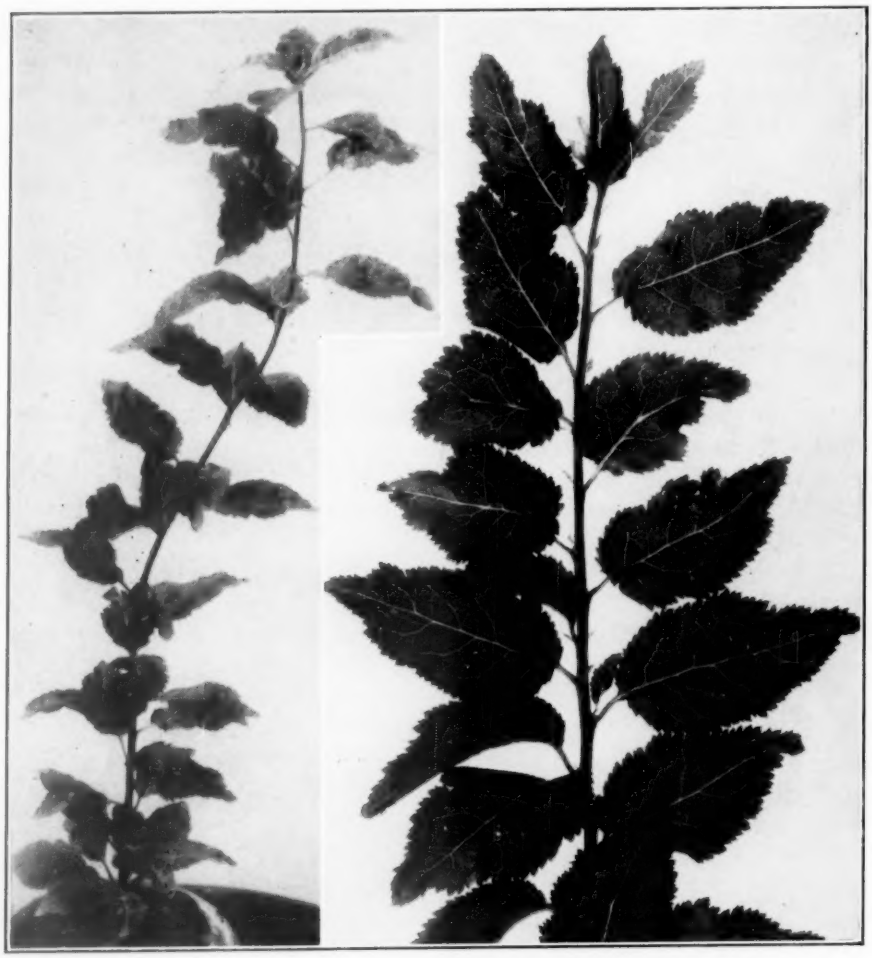

FIG. 1. Myrobalan mottle: left, young seedling plant; right, close-up view of foliage by transmitted light.

(5 mm. ±) on the stems are usually oval and may become necrotic although this is unusual. Every rootstock collection brought together thus far has had some asteroid spot. For example, in the 22 collections made in 1942 its presence ranged from occasional to 90 per cent. When symptoms were severe, root stock growth was stunted. In several experiments the percentages of plum buds surviving and growing the following season on severely

affected seedlings were considerably below, frequently less than 50 per cent, that in normal seedlings. The stem growth from Italian buds placed on affected plants was frequently stunted and symptoms on the foliage and stems were similar to those on the rootstock. On affected roots the annual growth from Italian buds was less than 2 feet in some cases, compared to 5 or 6 feet of growth on apparently normal roots.

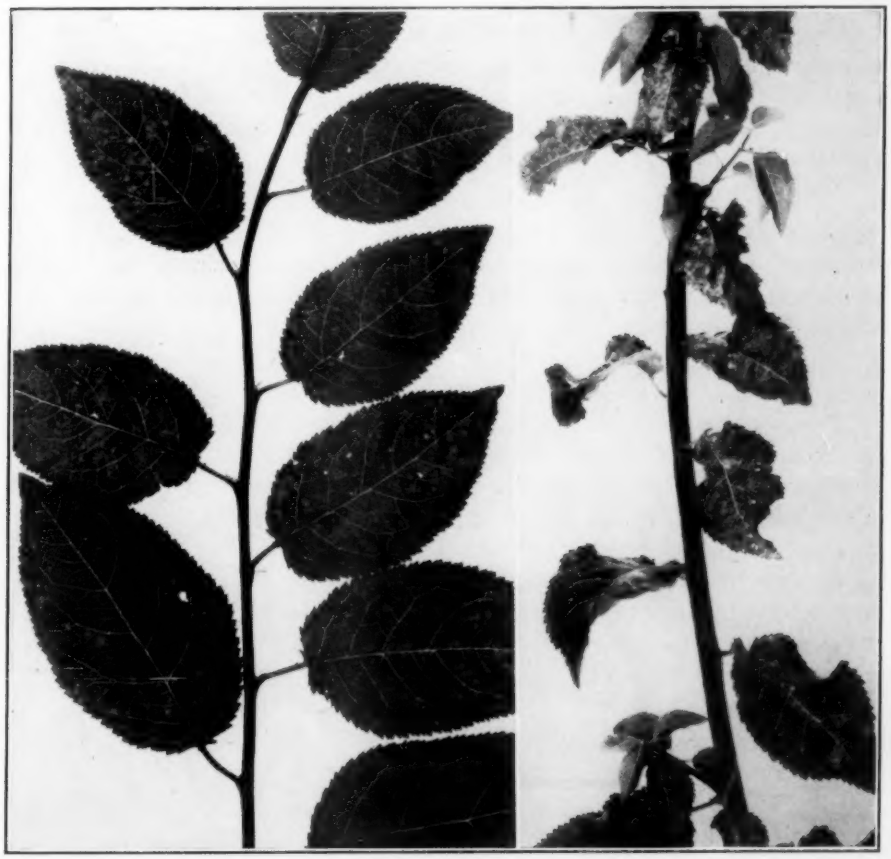

FIG. 2. Asteroid or chlorotic spot. On left, close-up of foliage in beginning stages showing few to many spots. On right, the advanced stage of spotting and distortion of the foliage accompanied by chlorotic spotting of the stem. Photographed by transmitted light.

Growing plants from seed in the greenhouse has practically excluded the chlorotic spot symptoms. However, since greenhouse temperatures run higher than those outdoors in spring and summer the possibility of temperature masking symptoms has not been excluded (2). When plants which showed symptoms outdoors in 1941, 1942, and 1943 were forced in the greenhouse the following winter no symptoms developed except on two plants which showed a few spots on the first growth made in early March. On neither the growth resulting from grafting with scions inserted during dor-

mancy nor from buds inserted during the growing season were symptoms later expressed at greenhouse temperatures. Grafting greenhouse seedlings with buds from severely affected plants in the field during the growing season has always given negative results. Symptomless seedlings grown from seed in the greenhouse when transferred to the field sometimes developed symptoms. At present the true cause of asteroid or chlorotic spot remains unknown.

The possibility that a certain insect may be the causative agent through injection of a toxin in its feeding operations has been probed. Thus far convincing evidence has not been obtained for or against this possibility. All attempts to transmit this abnormality to peach and other Prunus species have been negative.

DISCUSSION

No relationship seems to exist between myrobalan mottle and prune mottle (3) with which it might be confused. No relationship seems to exist between myrobalan asteroid or chlorotic spot and the necrotic asteroid spot virosis of peach reported from California (1).

SUMMARY

Two abnormalities, mottle and asteroid or chlorotic spot, are described on myrobalan plum (*Prunus cerasifera*) root-stocks.

Myrobalan mottle, which commonly occurs in nursery plantings but affects only a small percentage of the seedlings, is a seedborne, bud-perpetuated abnormality presumably of genetic origin.

Asteroid or chlorotic spot which is of unknown etiology has been frequently encountered in nursery plantings where as high as 90 per cent of the seedlings may show symptoms. Severe symptoms result in stunting of both rootstock and scion growth.

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THE PREVALENCE OF SEPTORIA ON CEREAL SEED IN CANADA[1]

J. E. MACHACEK[2]

(Accepted for publication, August 3, 1944)

In the autumn of 1937, the writer isolated from surface-sterilized Manitoba-grown wheat seed several cultures of a pale buff-salmon fungus. Pure cultures of this fungus produced, after an interval of several weeks, a few pycnidia on or near the surface of the culture. Spores typical of *Septoria nodorum* Berk. were formed in these pycnidia. Some of the wheat glumes present in the seed sample also carried pycnidia of *S. nodorum*.

In 1939, and in the following four years, a large number (6,183) of wheat, oats, and barley seed samples, collected from all the provinces of Canada, were subjected to a mycological examination. A part of the results from this investigation has already been published.[3] From this seed, *Septoria* frequently was isolated. It was present most often in wheat seed and least often in oats seed. The relative prevalence of *Septoria* in these crops, as indicated by a plating-out test of surface-sterilized seed on potato-sucrose agar, in each of the eight seed-inspection districts of Canada is given in table 1, and the appearance of the fungus, as it emerges from surface-sterilized wheat seed, is shown in figure 1, A.

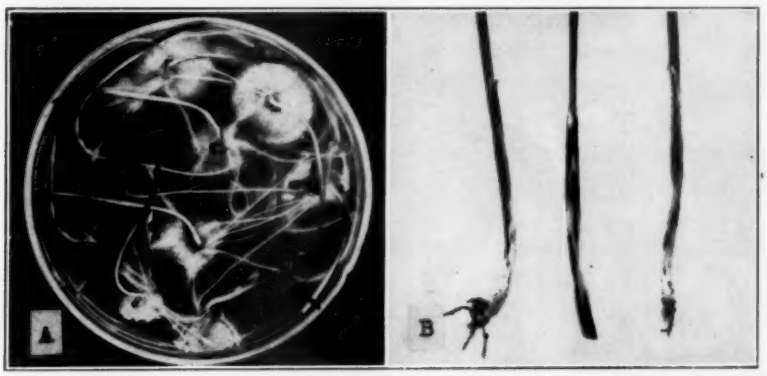

FIG. 1. Seed-borne *Septoria nodorum*. A. Fungus (light-colored colonies) emerging from surface-sterilized wheat seed. B. Lesions on coleoptiles of seedlings from infected wheat seed. Photo by A. M. Brown.

When wheat seed infected with *Septoria* was planted in nonsterile soil or sand in the greenhouse, many of the resulting seedlings showed elongate brown flecks or streaks on their coleoptiles (Fig. 1, B). With a few heavily infected lots of seed, some of the seedlings were distorted and, in addition to

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[3] Greaney, F. J., and J. E. Machacek. Prevalence of seed-borne fungi in cereals in certain seed-inspection districts of Canada. Sci. Agr. 22: 419–437. 1942.

the lesions on the coleoptiles, bore a few small lesions on the leaves. *Septoria nodorum* was isolated from lesions on the coleoptiles and from lesions on the leaves. Lesions on the coleoptiles also developed in the field. When infected seed of oats and barley was planted in soil or sand in the greenhouse, the seedlings were free from lesions and were not distorted.

Coleoptile and leaf lesions, as well as stem distortion, were prevented when infected wheat seed was treated with New Improved Ceresan at the rate of one-half ounce per bushel, or with other organic mercury dusts at the recommended rates.

TABLE 1.—*The relative prevalence of Septoria on the seed of wheat, oats, and barley in each of 8 seed-inspection districts of Canada during 1939–1943, and the percentage of infected seeds in the most severely infected seed samples in each district*

Inspection district	Crop	Mean percentage of infected seeds					Highest percentage of infected seeds				
		1939	1940	1941	1942	1943	1939	1940	1941	1942	1943
Maritime Provinces (Dist. 1)	Wheat	1.35	0.00	17.84	19.63	12.29	7	0	77	68	49
	Oats	0.20	0.00	0.04	0.03	0.05	1	0	1	1	1
	Barley	0.03	0.25	1.00	0.12	0.55	1	1	13	1	6
Quebec (Dist. 2)	Wheat	0.57	0.00	1.20	1.28	5.40	2	0	16	16	47
	Oats	0.00	0.04	0.00	0.04	0.11	0	1	0	1	2
	Barley	0.14	0.13	0.00	0.12	0.06	2	1	0	1	1
Eastern Ontario (Dist. 3)	Wheat	0.00	0.00	0.63	1.16	0.94	0	0	16	8	24
	Oats	0.12	0.00	0.00	0.00	0.11	1	0	0	0	1
	Barley	0.00	0.00	0.04	0.00	0.48	0	0	1	0	14
Western Ontario (Dist. 4)	Wheat	0.05	0.06	0.80	0.60	0.68	1	1	7	3	5
	Oats	.....	0.00	0.00	0.00	0.00	.....	0	0	0	0
	Barley	.....	0.00	0.11	0.00	0.16	.....	0	2	0	2
Manitoba (Dist. 5)	Wheat	0.02	0.07	0.01	0.74	1.09	2	3	2	12	8
	Oats	0.10	0.01	0.00	0.12	0.49	1	1	1	2	4
	Barley	0.01	0.06	0.00	0.50	1.09	1	3	0	10	21
Saskatchewan (Dist. 6)	Wheat	0.03	.....	0.06	1.16	1.27	4	.....	1	5	12
	Oats	0.03	.....	0.06	0.04	0.43	1	.....	1	1	4
	Barley	0.04	.....	0.13	1.88	5.02	1	.....	2	12	23
Alberta (Dist. 7)	Wheat	0.10	.....	0.50	1.40	0.94	6	.....	3	7	7
	Oats	0.00	.....	0.00	0.20	0.68	0	.....	0	5	10
	Barley	0.16	.....	0.36	2.34	3.96	5	.....	5	23	23
British Columbia (Dist. 8)	Wheat	0.06	.....	0.00	0.66	0.63	1	.....	0	6	3
	Oats	0.12	.....	0.00	0.00	0.11	1	.....	0	0	2
	Barley	0.00	.....	0.00	0.09	0.45	0	.....	0	1	2

In seed from the Maritime Provinces and from Quebec, more disease was present in wheat than in barley (Table 1). The opposite situation was found in the seed from Saskatchewan and Alberta, particularly in 1942 and 1943. This finding suggested the presence of two specialized strains or species of *Septoria* in the seed, one of which was more abundant in Eastern Canada and attacked primarily wheat while the other was more abundant on barley in Western Canada than on wheat.

A comparison of a large number of cultures derived from seed produced in both regions showed that the cultures from Eastern Canada generally

produced pycnidia more abundantly than did those from Western Canada and that the surface mycelium of the former cultures was generally less cottony than that of the latter. During the early stage of growth, however, it was impossible to distinguish with certainty the two types of cultures.

A large number of cultures, derived from seed produced in both areas, were submitted to Dr. T. Johnson of this laboratory, for a critical examination. He found that the cultures from Western Canada, as a rule, produced pycnospores distinctly longer and thicker than those formed by the cultures from Eastern Canada. The size and appearance of the spores from the Western Canada cultures excluded the possibility that the fungus was either *Septoria tritici* Desm. or *S. passerini* Sacc., and tests with suitable hosts excluded *S. avenae* Frank. The majority of the cultures from Eastern Canada were identified as *S. nodorum*.

It is difficult to understand why *Septoria nodorum* has not been recognized earlier as a common seed-borne, disease-producing organism. From a study of cereal seed during the past five years, the fungus appears to be abundantly present in Eastern Canadian wheat seed—one seed sample from the 1942 crop, when sown in soil, gave rise to seedlings of which 88 per cent were lesioned. Rosen[4] implied that *S. nodorum* was seed-borne, but did not actually prove it. The tardiness with which most of the cultures from seed sporulate probably has been one of the reasons why the occurrence of the fungus on seed has attracted so little attention.

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SALTS AS ANTIDOTES TO COPPER IN ITS TOXICITY TO THE CONIDIA OF SCLEROTINIA FRUCTICOLA[1]

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(Accepted for publication August 10, 1944)

The conidia of *Sclerotinia fructicola* are very sensitive to the fungicidal action of copper. Lin (4) has reported, however, that the toxicity of copper sulphate to these spores may be strikingly decreased by any one of several inorganic salts. The data presented here confirm the existence of Lin's "copper antidoting" phenomenon and provide certain new information concerning it.

High germination of the conidia of *S. fructicola* brought about by addition of an antidoting salt to a copper-sulphate-containing medium has been found to be associated with a decrease in the amount of copper absorbed by the spore. The protective phenomenon may be observed also in the respiratory behavior of the spores. Data on these and related points are discussed from the standpoint of the physiology of spore germination and the mechanism of the toxic action of copper.

MATERIALS AND METHODS

Three different measurements have been made on the conidia of *Sclerotinia fructicola*—percentage germination, respiratory rate, and copper absorption. Spore-germination experiments were in Pyrex Petri dishes containing glass-distilled water, copper sulphate, glucose, spores, and in some cases, an antidoting salt. Spores for these experiments were obtained by adding a little water to 10–14-day-old Petri-dish cultures on potato-dextrose agar, dislodging the spores with a camel's hair brush, filtering through Kleenex to remove hyphal fragments, and washing the spores by three successive centrifugations in 50-ml. portions of glass-distilled water. Spore germination counts were made after 20 hours at 26° C.

In the spore germination experiments (Table 1), spore concentrations were adjusted to approximately 25,000 spores per ml. In the copper absorption and respiration experiments, the concentrations ranged from 300,000 to 1,000,000 per ml. In early experiments, a Levy counting chamber was used to determine spore concentrations. A more rapid method adopted in later experiments utilized the measurement of light absorption by the suspensions in a photoelectric colorimeter.

In determining the rate of oxygen consumption by the spores, the Fenn (1) micro-respirometer was used. This instrument consists essentially of a small glass experimental vessel which is closed to the atmosphere when in operation but connected with a graduated capillary containing an index drop which is free to move in response to the consumption of oxygen by the

[1] Presented to the faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, November, 1943.

[2] The writer wishes to acknowledge his indebtedness to Prof. V. L. Frampton for helpful comments during the course of this study.

respiring tissue. The carbon dioxide of respiration is absorbed by KOH in an inset in the experimental vessel. The micro-respirometers used had a volume of 11 ml. in the experimental vessels and capillary volumes close to 3.5 mm.^{3} per linear centimeter. They were rocked in a water bath held at a constant temperature of 26° C.

Except where otherwise indicated, copper was determined colorimetrically by the use of sodium diethyl dithiocarbamate. The reagent has been studied by Greenleaf (3) and others. In determining copper in the supernatant fluid after germination, 5 ml. of the supernatant fluid was pipetted into the 10-ml. absorption cell of a Cenco-Sheard-Sanford photelometer. To this was added 1 ml. of a 0.01 per cent carbamate reagent, and glass-distilled water to a total volume of 10 ml. The concentration of copper was determined from a previously prepared calibration curve. Determination of copper by this method was very satisfactory over a range of concentra-

TABLE 1.—*Effect of single salts in antidoting the inhibitory effect of copper sulphate on germination of the conidia of Sclerotinia fructicola*

Salt conc.	Percentage germination in 4×10^{-7} M $CuSO_4$ plus 0.01 per cent glucose and:		
	$MgSO_4$	$CaCl_2$	KCl
0	1.2	0.8	2.9
10^{-5} M	54.0	31.0	3.9
10^{-4} M	67.0	62.0	2.6
10^{-3} M	78.0	83.0	3.9
10^{-2} M	.....	.....	59.0

tions from 10^{-5} M to 10^{-4} M, *i.e.*, from about 0.6 gamma per ml. to 6 gamma per ml. Copper determinations with potassium ethyl xanthate were according to the same procedure used with the dithiocarbamate.

In determinations of the copper content of the conidia themselves, the spores were first ignited in porcelain crucibles at a temperature below red heat in a muffle furnace. After the crucibles had cooled, a small amount of concentrated nitric acid was added; this was evaporated off on a hot plate and replaced by about 2 ml. of concentrated ammonia. After 4 or 5 hours, water and carbamate reagent were added and the determination completed.

Water used in the germination medium and in respiration and copper absorption determinations was prepared by redistillation of ordinary distilled water in a Pyrex glass still. The glucose used was prepared by recrystallization of Baker's reagent grade glucose. All other chemicals were of reagent grade. All glassware was cleaned in dichromate-sulphuric acid cleaning solution and rinsed thoroughly in tap water, distilled water, and glass-distilled water before use.

EXPERIMENTAL RESULTS

Table 1 presents the results of 3 spore-germination experiments and demonstrates the protective action of salts in the presence of copper sulphate.

TABLE 2.—*Magnesium sulphate protects conidia of Sclerotinia fructicola against poisoning of respiration by copper sulphate; antagonizes effect of copper sulphate on germination*

Hour	Cubic mm. O_2 consumption per million spores		
	Control	In 2×10^{-4} M $CuSO_4$	In 2×10^{-4} M $CuSO_4$ plus 2×10^{-2} M $MgSO_4$
1st	7.87	3.18	5.01
2nd	9.06	1.68	4.37
3rd	9.26	2.05	4.15
4th	9.65	1.49	4.82
5th	9.70	1.14	4.95
6th	9.16	0.86	5.00
7th	9.11	0.93	5.00
	Percentage germination after 8 hr.		
	95	0	40

Each germination plate contained 4×10^{-7} M copper sulphate and 0.01 per cent glucose; the salt concentrations ranged from zero to as high as 10^{-2} M for the KCl. It will be noted that mol for mol KCl was less effective as a protective agent than either $MgSO_4$ or $CaCl_2$.

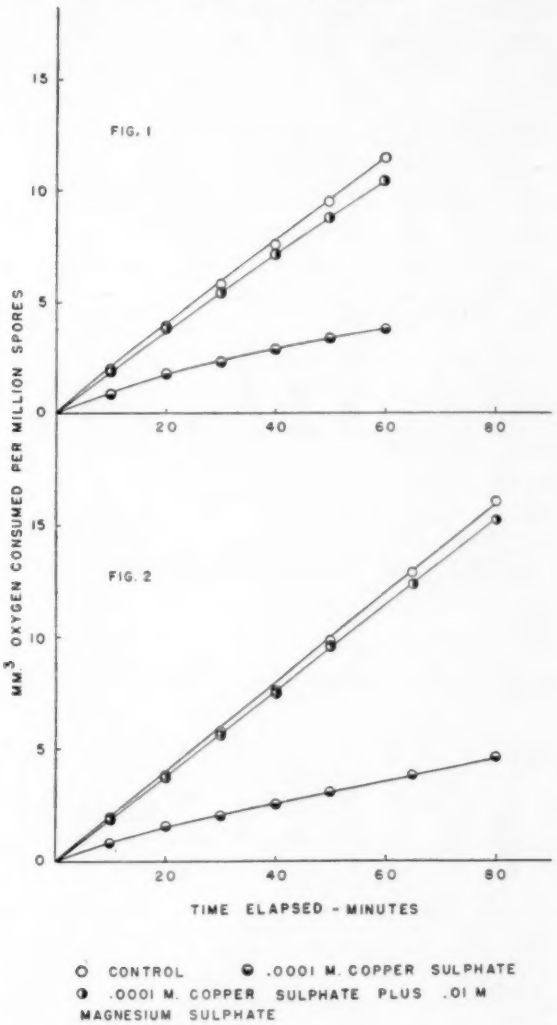
Figures 1 and 2 and table 2 present the protective phenomenon as it may be observed in the respiratory rates of the spores. Equal amounts of a conidial suspension in 1 per cent glucose were placed in each of three Fenn (1) micro-respirometer vessels. Copper sulphate was added to one vessel and copper sulphate plus magnesium sulphate to a second vessel, while the third vessel was left as a control, containing neither copper sulphate nor added salt. Magnesium sulphate protects the conidia against respiratory poisoning by copper sulphate. Table 3 shows that calcium chloride exerts a similar protective action.

When supplied in suitable concentration, calcium chloride or magnesium sulphate will partially, or in some cases almost completely, prevent the ab-

TABLE 3.—*Calcium chloride protects conidia of Sclerotinia fructicola against poisoning of respiration by copper sulphate; antidotes the effect of copper sulphate on germination*

Hour	Cubic mm. O_2 consumption per million spores		
	Control	In 2×10^{-4} M $CuSO_4$	In 2×10^{-4} M $CuSO_4$ plus 2×10^{-2} M $CaCl_2$
1st	9.66	1.70	6.17
2nd	8.19	1.72	5.75
3rd	8.00	1.63	6.62
4th	9.95	1.17	8.31
5th	9.49	0.85	8.06
6th	9.39	1.03	7.31
	Percentage germination after 18 hr.		
	93	0.02	87

sorption of copper from a copper sulphate solution by conidia of the brown-rot fungus. This fact is clearly apparent from the data in figure 3. The experiments whose results are shown in figure 3 were carried out by adding to each of a pair of Pyrex Petri dishes 5 ml. of a spore suspension in 2 per cent glucose, then 1 ml. of 0.1 M $CaCl_2$ (or $MgSO_4$) to one of the plates, and



FIGS. 1 and 2. Magnesium sulphate protects conidia of *Sclerotinia fructicola* against respiratory poisoning by copper sulphate.

finally 1 ml. of 10^{-3} M $CuSO_4$ and water to a total volume of 10 ml. to each plate. After 20 hours, the spores were centrifuged out of suspension and copper determinations made with the dithiocarbamate reagent. In experiments C–1 to C–10 inclusive, copper determinations were made on the supernatant fluid remaining over the spores after centrifuging the suspensions.

In experiments C–11 to C–14 and M–11 and M–12, copper analyses were made on the spores themselves. Spore germination in the copper absorption experiments was in all cases very much higher in the presence of the antidoting salt than in its absence. The germination figures for experiments C–1 to C–10 were 91, 95, 90, 95, 90, 92, 80, 64, 90, and 88 per cent for the "plus salt" plates and 2, 1, 1, 2, 0, 1, 0, 0.5, 1, and 0 per cent for the corresponding "minus salt" plates; similarly in the M series, germination percentages in the "plus salt" plates were 92, 96, 93, 84, 86, 91, 90, 92, 86, and 89

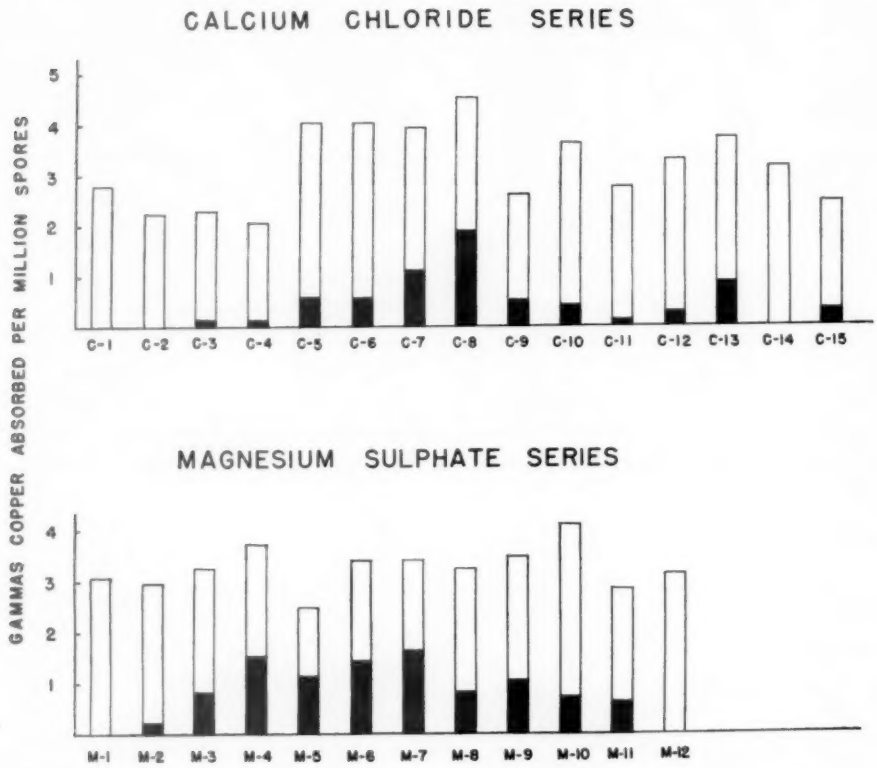


FIG. 3. Calcium chloride and magnesium sulphate depress the absorption of copper by conidia of *S. fructicola* from a copper sulphate solution. Total bar shows copper absorbed in absence of salt, blackened portion the same in the presence of salt.

as compared with 0, 1, 0, 0, 0, 0, 1, 1, 2, and 1 in the corresponding "minus salt" plates.

Salt depression of copper absorption may be observed after a very short absorption interval. In two 10-minute experiments, carbamate analysis of the supernatant fluid resulted in values of 0.36 and 0.45 gammas of copper absorbed per million spores in the presence of 10^{-2} M $CaCl_2$ and 10^{-4} M $CuSO_4$ as compared with 2.50 and 2.30 gammas absorbed in the presence of the copper sulphate alone. Similar experiments with $MgSO_4$ yielded values of 0.49 and 0.51 gammas absorbed in the presence of the 10^{-2} M $MgSO_4$ and

10^{-4} M $CuSO_4$ as compared with 1.59, 2.39, and 3.12 gammas absorbed in the absence of the protective salt. The 10-minute experiments differed from those of figure 3 in that no glucose or other carbon or energy source was supplied. The phenomenon of salt depression of copper absorption is thus rapid and not dependent on an external energy source for the spores. In the 10-minute experiments, the Petri dishes were rocked gently so that diffusion of copper in the solution would not limit the rate of copper absorption.

Experiments early in the course of the work had shown that neither $CaCl_2$ nor $MgSO_4$ interfered in the carbamate determination of copper; it was easily demonstrated further that when a known amount of copper sulphate was added to a supernatant fluid recovered from a spore suspension which had germinated in the presence of copper sulphate and either $CaCl_2$ or $MgSO_4$, this known increment of copper could be closely checked by carbamate copper analysis. As a final check, however, experiments were

TABLE 4.—*Effect of potassium chloride on the absorption of copper from a copper sulphate solution (10^{-4} M) by conidia of Sclerotinia fructicola*

Exp. No.	Molarity of KCl	Copper absorption and percentage germination with KCl			
		Present		Absent	
		Gammas Cu per million spores	Per cent germination	Gammas Cu per million spores	Per cent germination
K–1	0.01	2.22	0	2.62	0
K–2	0.1	2.82	0	4.06	0
K–3	0.5	0.51	5	3.10	0
K–4	1.0	0.0	78	4.36	0

carried out in which an entirely different reagent was used for determining copper, namely potassium ethyl xanthate. In three 10-minute absorption experiments, using 10^{-2} M $CaCl_2$ as antidote to 10^{-4} M $CuSO_4$, the copper absorption was 8.10, 4.49, and 4.14 gammas per million spores in the absence of the salt and essentially zero, or below the limit of determination, in its presence. In similar experiments with 10^{-2} M $MgSO_4$, the absorption values were 6.89 and 8.86 gammas in the absence of salt and zero in its presence.

Table 1 shows that the minimum effective ratio of KCl to $CuSO_4$ which is required for antidoting action to be observed in spore germination differences is very much higher than the same ratio for $CaCl_2$ or for $MgSO_4$. Similarly, the minimum effective ratio of KCl to $CuSO_4$ required to influence copper absorption is very high. Results with a range of concentrations of KCl are in table 4. Germination in the higher concentrations of KCl is somewhat slower than ordinary, but the germ tubes are normal in appearance.

DISCUSSION

The absorptive capacity of the conidia of *Sclerotinia fructicola* for copper in terms of copper per unit volume of spore is high. It is thus possible for conidia of this fungus in contact with a dilute copper sulphate solution to

absorb a large proportion of the copper from the surrounding liquid medium. This fact, in conjunction with the circumstance that the actual quantity of living material per unit volume in spore suspensions of the density ordinarily used in spore germination work is extremely low, offers at least a partial explanation of the extraordinarily low concentrations of copper which are effective in preventing germination of the conidia of *S. fructicola*. In experiment C–1, for example, the total number of gammas of copper present in the "minus salt" dish was 62.5. At the end of the experiment 19.2 gammas of copper had moved into the spores, whose total volume was only about 0.22 per cent of the total volume of the suspension. The concentration of copper per unit volume of spores at the end of the experiment was thus 1,930 times as great as in the external medium. Similar calculations for experiments C–1 to C–10 and M–1 to M–10 revealed that in some cases the concentration of copper in the spores was as much as 4,000 times as high as in the external medium.

When a salt presented singly to an organism inhibits its normal growth or activity but this inhibitory effect is diminished by addition of a second salt, the phenomenon observed is usually called ion antagonism. According to this definition, the copper-antidoting phenomenon here described is a case of ion antagonism. While much has been written concerning a relation between ion antagonism and ion absorption, actual experimental data on the point are very meager. The data here presented are thus of theoretical interest from the standpoint of the understanding of ion antagonism reactions in general.

While salts such as $MgSO_4$, $CaCl_2$, and KCl antidote the toxic action of copper toward the conidia of *S. fructicola*, it appears from the experiments here reported that these conidia do not require the addition of such salts or indeed of any other materials beside pure glucose and pure water to enable them to germinate in the absence of toxic materials. In this respect the author's results are at variance with those of McCallan and Wilcoxon (5) and those of Goldsworthy and Green (2). The cause of this difference in results has not been ascertained and might be difficult to prove. The possibility cannot be overlooked, however, that traces of toxic metals were present in the ordinary distilled water used by these investigators and that the function of orange juice or other natural decoctions necessary to secure high germination in the check plates was related to their content of heavy-metal-inactivating salts and proteins.

SUMMARY

Experiments are described which deal with the toxic action of copper sulphate on the conidia of *Sclerotinia fructicola*. It is shown that calcium chloride, magnesium sulphate, or potassium chloride may counteract or antidote the inhibitory effect of copper sulphate on the germination of these spores; this confirms the observations of Lin (4).

The copper-antidoting action of salts may be observed in the respiratory rates of the spores.

High germination of the spores brought about by the protective action of salts is associated with decreased copper absorption.

The spores of this fungus have a very high affinity for copper, being able to concentrate it within themselves from a very dilute solution.

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SPECIALIZATION OF PATHOGENICITY IN ERYSIPHE GRAMINIS ON POA AND ITS RELATION TO BLUEGRASS IMPROVEMENT[1]

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In a previous paper (1) the results of inoculation experiments with *Erysiphe graminis* DC. were reported in which fungus cultures from grasses in the tribe Hordeae infected only grasses in this tribe. Furthermore, each culture infected species of two or more genera, thus demonstrating that races of *E. graminis* are not necessarily restricted to the species of any one genus as had been previously supposed for the most part. The present paper reports the results of inoculation experiments with five cultures of the fungus from species of *Poa*.

Marchal (4) described the specialized variety, *Erysiphe graminis poae*, as infecting different *Poa* species, notably *P. annua*, *P. caesia*, *P. mutalensis*, *P. nemoralis*, *P. pratensis*, *P. serotinia*, and *P. trivialis*. Conidia from *P. annua* and *P. pratensis* did not infect *Hordeum vulgare*. Marchal inferred that *E. graminis poae* did not infect *Avena sativa*, *Bromus* species, *Hordeum vulgare*, *Secale cereale*, or *Triticum vulgare*.

Salmon (6) reported that conidia from *Poa pratensis* produced infection on *P. pratensis* and sub-infection on *Poa annua* and *P. nemoralis*. There was no infection of *Agropyron repens*, *Alopecurus pratensis*, *Avena sativa*, *Dactylis glomerata*, *Festuca elatior* var. *pratensis*, *F. arundinacea*, *F. heterophylla*, *Hordeum vulgare*, *Lolium perenne*, *L. temulentum*, *Phleum pratense*, *Secale cereale*, or *Triticum vulgare*.

Reed (5) states that conidia from *Poa pratensis* infected *P. compressa*, *P. nemoralis*, *P. pratensis*, and *P. trivialis* but did not infect *Avena sativa*, *Bromus mollis*, *Dactylis glomerata*, *Festuca elatior*, *Hordeum jubatum*, *H. vulgare*, *Lolium perenne*, *Phleum pratense*, *Secale cereale*, or *Triticum vulgare*. Reed also suggested that there may be more than one specialized race within *E. graminis poae*.

Unpublished notes taken by the writer in grass nurseries at Ann Arbor, Michigan, and Pullman, Washington, record several instances of mildew-susceptible accessions of *Poa* species remaining uninfected while growing in rows adjacent to heavily mildewed bluegrass plants of the same as well as of different species of *Poa*.

[1] Part of this investigation was made at the University of Michigan in studies leading to the degree of Doctor of Philosophy in Botany, and part at the Kentucky Agricultural Experiment Station. It is published by permission of the Director of the Kentucky Agricultural Experiment Station.

[2] The writer gratefully acknowledges the suggestions and criticisms given by Dr. E. B. Mains; seed of the various grasses supplied by many collaborators; the cooperation of Dr. George W. Fischer in sending cultures of the fungus, and the assistance of Mr. J. R. Swallen in checking the identity of the majority of the grasses used.

TABLE 1.—*The number of accessions of various grass species infected after inoculation with 5 cultures of Erysiphe graminis from 3 species of Poa*

Species tested[a]	Culture No. and source species				
	P. pratensis	*P. ampla*	*P. palustris*	*P. pratensis*	*P. pratensis*
	1	14	20	21	22
	Number of accessions infected[b]				
Aegilops crassa Boiss.	0/2	0/1	.....	.....	.....
A. cylindrica Host	.....	0/1	.....	0/1	.....
A. triuncialis L.	0/1	.....	.....	.....	.....
Agropyron caninum (L.) Beauv.	.....	.....	.....	0/1	.....
A. cristatum (L.) Beauv.	0/4	0/4	0/1	0/4	.....
A. desertorum (Fisch.) Schult.	0/1	0/1	0/1	0/1	.....
A. inerme (Scribn. and Smith) Rydb.	0/12	0/11	0/4	0/1	.....
A. intermedium (Host) Beauv.	0/1	.....	0/1	0/1	.....
A. repens (L.) Beauv.	0/1	.....	0/1	0/1	.....
A. semicostatum (Steud.) Nees	0/1	0/1	0/1	0/1	.....
A. sibiricum (Willd.) Beauv.	0/5	0/5	0/2	0/5	.....
A. smithii Rydb.	0/1	0/1	0/1	0/1	.....
A. spicatum (Pursh) Scribn. and Smith	0/13	0/11	0/2	0/12	.....
A. striatum (Steud.) Nees ex Hook.	.....	0/1	.....	0/1	.....
A. subsecundum (Link) Hitchc.	0/1	0/1	.....	0/1	.....
A. trachycaulum (Link) Malte	0/13	0/14	0/3	0/13	.....
Agrostis alba L.	0/4	0/4	0/4	0/4	0/5
A. exarata Trin.	0/1	0/1	0/1	0/1	0/1
A. hiemalis (Walt.) B.S.P.	.....	.....	.....	0/1	.....
A. palustris Huds.	0/1	0/1	0/1	0/1	0/1
A. scabra Willd.	.....	.....	.....	.....	0/1
A. spica-venti L.	.....	0/1	.....	0/1	.....
A. stolonifera L.	.....	0/1	.....	.....	.....
Alopecurus aequalis Sobol.	.....	0/1	.....	0/1	0/1
A. pratensis L.	.....	0/3	0/1	0/2	0/2
Anthoxanthum odoratum L.	.....	.....	.....	.....	0/1
Arrhenatherum elatius (L.) Mert. and Koch	0/1	0/1	0/1	0/1	0/1
Avena sativa L.	0/1	0/1	0/1	.....	.....
Beckmannia erucaeformis (L.) Host	0/1	0/1	0/1	0/1	0/1
B. syzigachne (Steud.) Fernald	0/1	0/1	0/1	1/1	0/1
Bromus arvensis L.	0/2	0/2	.....	0/2	.....
B. brizaeformis Fisch. and Mey.	0/1	0/1	.....	0/1	.....
B. carinatus Hook. and Arn.	.....	0/2	0/2	0/2	.....
B. catharticus Vahl	0/3	0/3	.....	0/3	.....
B. commutatus Schrad.	0/1	0/1	.....	0/1	.....
B. inermis Leyss.	0/6	0/6	0/2	0/6	.....
B. japonicus Thunb.	0/2	0/2	.....	0/2	.....
B. macrostachys L.	0/2	0/2	.....	0/2	.....
B. macrostachys var. *lanuginosus* Boiss.	0/1	0/1	.....	0/1	.....
B. marginatus Nees	0/7	0/8	0/2	0/8	.....
B. mollis L.	0/2	0/2	.....	0/2	.....
B. polyanthus Scribn.	0/1	0/1	.....	0/1	.....
B. purgans L.	0/2	0/2	.....	0/2	.....
B. rigidus Roth	0/1	0/1	.....	0/1	.....
B. rubens L.	0/1	0/1	.....	0/1	.....
B. secalinus var. *velutinus* (Schrad.) Koch	0/1	0/1	.....	0/1	.....
B. squarrosus L.	0/1	0/1	.....	0/1	.....
B. tectorum L.	0/1	0/1	.....	0/1	.....

TABLE 1—(*Continued*)

Species tested[a]	Culture No. and source species				
	P. pratensis	*P. ampla*	*P. palustris*	*P. pratensis*	*P. pratensis*
	1	14	20	21	22
	Number of accessions infected[b]				
B. tectorum var. *glabratus* Spenner	0/1	0/1	.....	0/1	.....
B. vulgaris (Hook.) Shear	0/1	0/1	.....	0/1	.....
Dactylis glomerata L.	0/8	0/8	0/8	0/8	0/8
Deschampsia caespitosa (L.) Beauv.	1/1	0/1	0/2	0/2	0/2
D. danthonioides (Trin.) Munro	.....	1/1	.....	1/1	0/1
Elymus canadensis L.	0/5	0/4	0/2	0/5	.....
E. condensatus Presl	0/4	0/4	0/1	0/4	.....
E. dahuricus Turcz.	0/1	0/1	0/1	0/1	.....
E. glaucus Buckl.	0/4	0/3	.....	0/3	.....
E. junceus Fisch.	0/1	0/1	.....	0/1	.....
E. sibiricus L.	.....	0/1	.....	.....	.....
E. triticoides Buckl.	0/1	0/1	0/1	0/1	.....
E. villosus Muhl.	.....	0/1	.....	.....	.....
E. virginicus L.	0/3	0/4	.....	0/4	.....
E. virginicus var. *glabriflorus* (Vasey) Bush	0/1	0/1	.....	.....	.....
E. virginicus var. *intermedius* (Vasey) Bush	.....	0/1	.....	.....	.....
Festuca elatior L.	0/1	0/1	.....	0/1	0/1
F. elatior var. *arundinacea* (Schreb.) Wimm.	0/1	0/1	.....	0/1	0/1
F. gigantea (L.) Vill.	0/1	.....	.....	0/1	0/1
F. idahoensis Elmer	.....	0/1	0/1	1/1	0/1
F. obtusa Spreng.	.....	0/1	.....	.....	.....
F. occidentalis Hook.	.....	1/1	.....	1/1	.....
F. octoflora Walt.	.....	1/1	.....	1/1	0/1
F. ovina L.	.....	0/1	.....	.....	.....
F. rubra L.	1/1	0/2	.....	0/1	0/1
F. rubra var. *commutata* Gaud.	1/1	0/1	.....	1/1	0/1
F. scabrella Torr.	.....	0/1	.....	.....	.....
F. thurberi Vasey	.....	1/1	.....	1/1	0/1
F. viridula Vasey	.....	0/1	.....	.....	.....
Holcus lanatus L.	0/1	0/1	.....	0/1	0/1
Hordeum bulbosum L.	.....	0/1	.....	.....	.....
H. gussoneanum Parl.	.....	0/1	.....	0/1	.....
H. jubatum L.	.....	.....	.....	0/1	.....
H. jubatum var. *caespitosum* (Scribn.) Hitchc.	0/1	0/1	.....	.....	.....
H. murinum L.	0/1	.....	.....	0/1	.....
H. nodosum L.	.....	.....	.....	0/1	.....
H. vulgare L.	0/2	0/2	0/2	0/2	.....
Hystrix patula Moench	.....	.....	0/1	.....	.....
Koeleria cristata (L.) Pers.	3/5	3/7	0/6	2/4	0/5
Lolium multiflorum Lam.	0/1	0/1	0/1	0/1	.....
L. perenne L.	0/1	0/1	0/1	0/1	.....
Milium effusum L.	.....	0/1	.....	.....	0/1
Phalaris arundinacea L.	1/1	0/1	0/1	0/1	0/1
Phleum pratense L.	2/3	0/3	0/3	3/3	0/3
Poa ampla Merr.	3/7	21/30	0/7	0/30	0/13
P. arachnifera Torr.	.....	0/1	.....	1/1	0/1
P. arctica R. Br.	1/1	1/1	.....	1/1	0/1
P. arida Vasey	.....	1/1	.....	0/1	0/1
P. bulbosa L.	1/1	2/4	0/1	0/4	0/6

TABLE 1—(*Continued*)

Species tested[a]	Culture No. and source species				
	P. pratensis	*P. ampla*	*P. palustris*	*P. pratensis*	*P. pratensis*
	1	14	20	21	22
	Number of accessions infected[b]				
P. canbyi (Scribn.) Piper	0/3	6/8	0/3	0/8	0/5
P. compressa L.	2/3	2/3	1/3	2/3	0/5
P. curta Rydb.	1/1	0/1	0/1	1/1	0/1
P. cusickii Vasey	1/1	1/2	1/1	2/2	1/1
P. epilis Scribn.	1/1	1/2	0/1	1/1	0/2
P. gracillima Vasey	1/2	3/3	0/2	1/3	0/2
P. interior Rydb.	.....	0/1	.....	0/1	0/1
P. juncifolia Scribn.	0/2	4/5	0/2	0/5	0/6
P. nemoralis L.	1/3	3/7	0/1	0/6	0/8
P. nervosa (Hook.) Vasey	.....	0/2	.....	0/2	0/2
P. nevadensis Vasey	2/3	6/6	0/3	0/6	0/4
P. palustris L.	1/2	2/6	4/4	2/7	2/4
P. pratensis L.	11/12	1/16	2/11	14/15	42/88
P. scabrella (Thurb.) Benth.	0/1	3/3	0/1	0/3	0/3
P. secunda Presl	2/3	6/6	0/3	0/6	1/8
P. sphondyloides Trin.	2/3	5/5	0/3	1/4	1/5
P. sterilis Bieb.	.....	0/1	.....	1/1	1/1
P. trivialis L.	.....	.....	.....	.....	1/2
Polypogon monspeliensis (L.) Desf.	.....	.....	0/1	0/1	0/1
Puccinellia distans (L.) Parl.	.....	1/1	0/1	1/1	0/1
Secale cereale L.	0/1	0/1	0/1	0/1	.....
Sitanion hystrix (Nutt.) J. G. Smith	0/1	0/1	0/1	0/1	.....
S. jubatum J. G. Smith	0/1	0/1	0/1	.....	.....
Trisetum flavescens (L.) Beauv.	.....	0/1	0/1	1/1	0/1
T. spicatum (L.) Richt.	.....	0/1	.....	1/1	0/1
Triticum aestivum L.	0/1	0/1	0/1	0/1	.....

[a] Hitchcock (2) was followed wherever possible for the nomenclature of the grasses.
[b] Numerator of fraction refers to number of accessions infected; denominator refers to total number of accessions tested. The term accession is explained under Materials and Methods.

MATERIALS AND METHODS

Selected groups of 398 accessions of the grass species listed in table 1 were used. The methods of handling fungus cultures and grasses have been described in detail in another paper (1). Grass seedlings were inoculated by atomizing their leaves with an aqueous suspension of fresh conidia and covering the inoculated plants with wet muslin for 24 to 48 hours. The classes of reaction used in recording notes were those of Mains and Dietz (3, p. 231).

The term "grass accession" in this paper refers to a numbered collection which represents a sample of the population of any one grass species. In most cases, these collections are genetically variable. Many of them were obtained from the Soil Conservation Service and represented seed produced from their collections grown in row nurseries. The specific identifications of these grass accessions were checked by the personnel of the Soil Conservation Service. Recognizing the possibilities of error both in previous identifi-

cations and in harvesting and preparation of nursery seed samples, a number of plants of all grass accessions used were grown to maturity. Representative samples of most accessions were sent to Mr. J. R. Swallen, Division of Plant Exploration and Introduction, U. S. Department of Agriculture, for verification. Likewise, careful personal attention was given to the gross morphology of both adult and seedling plants. The possibility of species mixtures was under constant surveillance, and particularly when unusual infection results were obtained. These precautions should give reasonable assurance of accuracy in regard to the specific identity of the grasses.

RESULTS

In table 1 are the general results of inoculating from 1 to many accessions of 116 species and 8 varieties of grasses with 5 cultures of *Erysiphe graminis* from 3 species of *Poa*. These results demonstrate that powdery mildew from *Poa* is not necessarily restricted to species of this genus. The large-scale negative results permit a clearer understanding of the host range of Poa mildews. The grass accessions listed as infected in table 1 include all cases where definite infection was recorded.

Distinction is made between resistant and susceptible types of reaction of grass accessions in table 2. The 38 accessions of various grass species listed in table 2 were chosen because their reactions are most significant in illustrating differences in the pathogenicity of the 5 mildew cultures and in the host range of Poa mildews. If only resistant types of reaction are found in grass accessions of certain grass species, these may be important clues to a more complete understanding of the host range, since other untested collections of the species may be very susceptible. This is especially true when a restricted number of collections of grass species are tested.

Culture 1. On Poa pratensis, from Ann Arbor, Michigan

Many species of *Poa* were infected by culture 1 and, in addition, *Deschampsia caespitosa*, *Festuca rubra*, *F. rubra* var. *commutata*, *Koeleria cristata*, *Phalaris arundinacea*, and *Phleum pratense*. The largest number of susceptible bluegrasses are among the related species of the *Pratenses* and *Epiles* groups of the genus *Poa*.[3] Culture 1 was neither as vigorous nor as destructive as cultures 21 or 22 also from *P. pratensis*.

Culture 21. On Poa pratensis, from Mandan, North Dakota

Culture 21 was collected by Dr. Roderick Sprague and the writer at the Northern Great Plains Field Station. This vigorous culture infected more accessions and species of *Poa* as well as more species of other genera than did culture 1. Besides bluegrasses, infection was produced on species of *Beckmannia*, *Deschampsia*, *Festuca*, *Koeleria*, *Phleum*, *Puccinellia*, and *Trisetum*. Susceptible collections of bluegrasses were primarily among the related species of the *Pratenses* group of the genus *Poa*.

[3] The taxonomic subsections of the genus *Poa* are taken from Hitchcock (2).

TABLE 2.—*Reactions of 38 accessions of grasses to 5 cultures of Erysiphe graminis from 3 species of Poa*

Grass accessions[a]	Culture No. and source species				
	P. pratensis	*P. ampla*	*P. palustris*	*P. pratensis*	*P. pratensis*
	1	14	20	21	22
	Types of reaction[b]				
Agrostis exarata 21	0	0	0	0	0
Agrostis palustris 22	0	0	0	0	0
Alopecurus pratensis 234	.....	0	.....	0	0
Arrhenatherum elatius 23	0	0	0	0	0
Avena sativa 369	0	0	0	0	.....
Beckmannia erucaeformis 11	0	0	0	0	0
Beckmannia syzigachne 12	0	0	0	2	0
Bromus carinatus 229	.....	0	0	0	.....
Dactylis glomerata 31	0	0	0	0	0
Deschampsia caespitosa 9	2–	.....	0	0	0
Deschampsia caespitosa 10	0	0	0	0	0
Deschampsia danthonioides 318	.....	2–	.....	2	0
Festuca idahoensis 250	.....	0	0	2	0
Festuca octoflora 323	.....	3+	.....	2+	0
Festuca rubra var. *commutata* 85	2	0	.....	4	0
Holcus lanatus 187	0	0	.....	0	0
Koeleria cristata 32	0	0	0	0	0
Koeleria cristata 33	3–	2+	0	2+	0
Koeleria cristata 34	2+	2–	0	0	0
Milium effusum 223	.....	0	.....	.....	0
Phalaris arundinacea 13	3–	0	0	0	0
Phleum pratense 15	3	0	0	2+	0
Phleum pratense 16	0	0	0	2–	0
Poa arachnifera 224	.....	0	.....	4	0
Poa canbyi 43	0	4	0	0	0
Poa compressa 49	4	2–	2+	2+	0
Poa compressa 50	4	0	0	4	0
Poa curta 51	4	0	0	4	0
Poa nevadensis 59	0	3	0	0	0
Poa palustris 57	2	0	3	0–1	0
Poa palustris 65	0	1+	3+	0	0
Poa pratensis 68	3	0	0	2	0–1
Poa pratensis 69	3	0	0	0	0
Poa pratensis 70	3	0	0	4	0–1
Polypogon monspeliensis 230	.....	0	0	0	0
Puccinellia distans 251	.....	4	0	4	0
Trisetum flavescens 228	.....	0	.....	2	0
Trisetum spicatum 329	.....	0	.....	2	0

[a] Accession numbers assigned to grass collections by the writer.

[b] Classes of reaction expressed according to the system of Mains and Dietz (3):

0—Highly resistant. Little or no mycelium. Chlorotic or necrotic spots on some hosts.

1—Very resistant. Slight to moderate mycelium. No sporulation. Chlorotic or necrotic spots may develop.

2—Moderately resistant. Moderate to abundant mycelium. Slight sporulation. Chlorotic or necrotic areas may develop.

3—Moderately susceptible. Moderate to abundant mycelium. Moderate sporulation.

4—Very susceptible. Abundant mycelium and sporulation.

—Indicates no test was made.

Culture 22. On Poa pratensis from Lexington, Kentucky

Culture 22 was collected from plants in a well-established sod where the development of mildew resulted in severe defoliation during the fall and early winter of 1942. In the spring of 1943 very little new growth appeared in the affected small area. Although the sod reached a fair development by midsummer, the loss of spring growth was substantial.

Culture 22 infected fewer bluegrass species than cultures 1 and 21. No infection was produced outside of the genus *Poa*. As with culture 21, infected species are largely in the *Pratenses* group of the genus *Poa*.

Culture 14. On Poa ampla from Pullman, Washington

Culture 14 was started from infected plants sent by Dr. George W. Fischer. It infected many collections of species of *Poa* and also *Deschampsia danthonioides., Festuca occidentalis, F. octoflora, F. thurberi, Koeleria cristata,* and *Puccinellia distans.* Bluegrass species showing the greatest susceptibility to this culture are among the *Scabrellae* and *Nevadenses* groups of the genus *Poa*.

Culture 20. On Poa palustris from Pullman, Washington

Only collections of *Poa palustris* were moderately or very susceptible to culture 20. Resistant types of reaction were recorded on one accession each of *Poa compressa* and *P. cusickii* and two accessions of *P. pratensis.* The fact that no other bluegrasses were infected among the many species tested indicates that this culture may be rather closely restricted to *P. palustris.*

The pathogenically different cultures may be easily distinguished on one accession each of four species of *Poa:*

P. canbyi 43 highly resistant
  P. curta 51 highly resistant
    P. palustris 65 highly resistant ..... Culture 22
    P. palustris 65 moderately susceptible ..... Culture 20
  P. curta 51 very susceptible
    P. pratensis 69 highly resistant ..... Culture 21
    P. pratensis 69 moderately susceptible ..... Culture 1
P. canbyi 43 very susceptible ..... Culture 14

DISCUSSION

Each of the 5 cultures studied was pathogenically distinct, so that this work is the first definite record of pathogenic specialization within *Erysiphe graminis* from *Poa.* Three different mildew races occurred on *P. pratensis,* and there are undoubtedly more yet to be isolated. Thus, strains of *P. pratensis* resistant to mildew in one area very likely will be infected in other areas where different races of *E. graminis* probably occur or in the same area upon the introduction of new mildew races.

Cultures 1, 21, and 22 from *P. pratensis* infected primarily related species of the *Pratenses* group; culture 14 from *P. ampla* principally species among

the *Scabrellae* and *Nevadenses* groups; and culture 20 from *P. palustris* almost exclusively species of the *Palustres* group of the genus *Poa*. This information will aid not only in understanding the host range and possible sources of inoculum of different races, but it also affects the evaluation of artificial and field tests of grass strains for mildew resistance. Mildew from *P. ampla* or *P. palustris* is probably of little consequence to the mildew problem in *P. pratensis*, since *P. pratensis* strains appear to be naturally resistant to those groups of mildew races. Likewise mildew races from *P. pratensis* appear not to be the ones responsible for damage to *P. ampla*, *P. palustris*, and related species.

Poa ampla, *P. palustris*, and *P. pratensis* and their relatives are growing in areas, especially the Pacific Northwest, where abundant perithecia form on certain species of *Poa* and where hybridization between races of mildew and segregations for pathogenicity probably are occurring. There is probably a natural selection of new races, screened out by the available host plants. Separation of Poa mildews into groups of races each of which infects a group of related grass species may be expected to parallel the groups of dominant species of *Poa* available as host plants. It is logical that mildew races that infect *Poa pratensis* should find closely related host species much more congenial than distantly related species. However, this does not exclude the possibility of genetic combinations within the fungus that will enable a mildew race to infect many species in several subgeneric groups of the genus *Poa*.

The mildews from *Poa* are not necessarily restricted to species of this genus. Several grasses can serve as common hosts for two or more different Poa mildew races and furnish a "breeding court" for hybridization between widely different races. *Puccinellia distans* 251 was very susceptible to both culture 14 from *Poa ampla* and culture 21 from *P. pratensis*. The opportunity for hybridization between different pathogenic races may be much reduced on certain grasses such as *Poa pratensis* and *P. palustris* since perithecia are seldom found on these species. Perithecia do form in abundance on *P. ampla*, *P. canbyi*, *P. juncifolia* and related species.

Two instances of natural mixtures of several mildew races differing greatly in pathogenicity on the same grass plants and many instances where several races could occur on the same grass were described in a previous paper (1). In *Erysiphe graminis* the production of new pathogenic races probably is limited only by the possible genetic combinations and by the sexual reproduction processes. Nothing has been reported concerning the nature of sexuality in *E. graminis*, but regardless of the exact nature of the processes involved, it is obvious that very pronounced segregations for pathogenicity have taken place.

Collections within species of *Poa* vary considerably in reaction to powdery mildew, a fact of importance in the selection and breeding of strains for mildew resistance. Bluegrass collections resistant to all five mildew cultures are: *Poa ampla*, 38, 42, 55, *P. canbyi* 44, and *P. juncifolia* 56. Mildew resis-

tance in these accessions is of considerable importance to agriculture in the Pacific Northwest where these bluegrass species constitute an important component of the native forage and where powdery mildew is a destructive disease on these species. *P. pratensis* 66 was highly resistant to cultures 1, 14, and 22, very resistant to culture 20, and moderately resistant to culture 21. It may be a desirable source of powdery mildew resistance, especially if it is resistant to other diseases.

In the genus *Poa* several very different modes of reproduction are known, and improvement methods are adjusted to meet the problems in individual species. Strains of *P. pratensis* have been isolated which are high-yielding when free of disease but which are susceptible to one or more destructive diseases. Improvement of such strains might be accomplished in several ways. Plants arising from occasional fertilization in apomictic strains and the possible segregations from both selfed and open-pollinated fertile strains might provide genetic recombinations resistant to one or more diseases to which the parents are susceptible. The high degree of apomictic reproduction in *Poa pratensis* has discouraged hybridization, and individual plant selection methods are largely utilized. However, successful hybrids between Texas bluegrass (*P. arachnifera*) and Kentucky bluegrass (*P. pratensis*) have been reported (7, pp. 1060, 1090) which compare favorably, especially in dry years, with outstanding selections of *P. pratensis*.[4] This cross was made primarily to produce a drought-resistant bluegrass. *P. arachnifera*, in addition to drought resistance, also carries resistance to certain diseases. One accession has been resistant to certain cultures of *Erysiphe graminis*, *Puccinia poae-sudeticae*, and *Helminthosporium vagans*. Therefore, it appears that the possibilities with this cross have not been fully exploited. The development of an outstanding strain of *P. arachnifera* should increase the value of hybrids with *P. pratensis*. If the hybrids should have disease resistance in addition to the agronomically desirable characters of the parent *P. pratensis* it would be an excellent method of overcoming susceptibility to disease in otherwise outstanding strains of *P. pratensis*. The fact that part of the hybrids are fertile suggests that this cross may greatly alleviate disadvantages residing with apomictic strains of *P. pratensis*. Fertility will permit the utilization of advantageous breeding methods that are not applicable to improvement of apomictic strains.

Striking resistance to all the cultures of powdery mildew was found in certain accessions of *Poa nemoralis* and *P. sphondyloides*. Preliminary studies also indicate that these species constitute a source of resistance to other diseases as well.

SUMMARY

Selected groups of 398 accessions of 116 species and 8 varieties of grasses in 28 genera were inoculated with five cultures of *Erysiphe graminis* obtained from three species of *Poa*.

[4] From Mr. E. Marion Brown, in correspondence.

Each of the five cultures is pathogenically distinct; thus, pathogenic specialization is recorded for the first time in *E. graminis* from *Poa*. Three of the cultures infected grasses outside the genus *Poa*, although each culture infected principally *Poa* species in the subgeneric group embracing the species on which it was obtained. Three different pathogenic races of powdery mildew were isolated from *P. pratensis* from three widely separated localities.

Several economic as well as wild species of *Poa* have been notably resistant to all five cultures.

Two advantages of hybridization between *Poa arachnifera* and *P. pratensis* in improvement of Kentucky bluegrass are (1) the possibility of obtaining disease resistance from *P. arachnifera*, and (2) the production of fertile hybrids from apomictic *P. pratensis* parents, thus permitting utilization of plant breeding methods not applicable to improvement of apomictic strains.

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HYPOXYLON PRUINATUM AND ITS PATHOGENESIS ON POPLAR[1]

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INTRODUCTION

The diseases of poplar have come into prominence because of the recently recognized value of poplar[3] in the forest economy of the North Central Region of the United States. The wood of these trees is used extensively for boxwood, paper pulp, and excelsior. The trees are valuable in forestry as a green-leaf firebreak planting, as a nurse crop for conifers, and as a source of deciduous leaves to help maintain soil fertility (15). No other trees seem so well suited for fulfilling such needs. A continuous supply of poplars can be maintained because they are fast-growing trees, are well adapted to much land that is submarginal for agriculture, are light-seeded and come in rapidly after a forest fire, and in many cases can be propagated vegetatively. Certain diseases, however, tend to offset these advantages.

The poplar canker, caused by *Hypoxylon pruinatum* (Kl.) Cke., was first described in the United States by Povah (10) and in Wisconsin by Kaufert (6). Its prevalence in Michigan, New York, Maine, and Ontario, Canada, has been reported by other workers (1, 10, 13). The incidence of the canker in several national forests in the Lake and Central States was studied by Lorenz and Christensen (8), who indicate that the canker is the most important poplar disease in the Lake States.

The value of the host and the importance of the disease have warranted further study of the canker, the results of which are recorded in this paper.

OCCURRENCE OF THE DISEASE

The prevalence of Hypoxylon canker on poplar in Wisconsin was studied during the summers of 1941, 1942, and 1943. Twenty-six field study plots, each $\frac{1}{10}$ acre in size, were established in 8 counties: 4 plots in Bayfield, 2 in Douglas, 4 in Iron, 2 in Marinette, 5 in Oneida, 4 in Vilas, 3 in Washburn, and 2 in Waushara. Every poplar in each plot was tallied by diameter class and was examined for Hypoxylon canker. The canker infection ranged from 0 per cent to 53 per cent with an average of 24 per cent. The site index (height of the average dominant tree at 50 years of age) for each plot was

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

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Acknowledgment is made to the Wisconsin Conservation Department for its cooperation and encouragement in this investigation. The writer is indebted to C. M. Christensen, Carl Hartley, Frank Kaufert, and C. A. Richards for advice concerning various phases of the work; to A. J. Riker for help and encouragement during the course of this study; and to Eugene Herrling for assistance in preparing the illustrations.

[3] Unless otherwise stated, the term "poplar" in this paper refers to *Populus tremuloides* Michx. and to *P. grandidentata* Michx.

determined by the method described by Kittredge and Gevorkiantz (7). The summaries of the cruise records are in tables 1 and 2.

In these data the prevalence of the disease was highest on the poorest site surveyed (Table 1, site index 40) and was lowest on the best site surveyed (Table 1, site index 80). This confirms earlier reports. While the reason for this was not determined, it is suggested that on the good sites the rate of escape, the disappearance of infected trees, and the resistance of the trees were greater than on the poor sites.

TABLE 1.—*Incidence of infection with Hypoxylon pruinatum by site index classes*[a]

Site index class	Trees examined	Trees with Hypoxylon canker
	Number	*Per cent*
40	168	51
50	112	24
60	716	22
70	408	22
80	178	4

[a] A portion of these figures is used through the courtesy of Dr. E. E. Honey, Emergency Plant Disease Prevention Project.

TABLE 2.—*Incidence of infection with Hypoxylon pruinatum by tree size (d.b.h.)*[a]

D.b.h.[b]	Trees examined	Trees with Hypoxylon canker
Inches	*Number*	*Per cent*
0–1	253	5
1–2	153	27
2–3	222	31
3–4	218	44
4–5	173	33
5–6	173	31
6–7	125	28
7–8	79	29
8–9	32	12
9–10	18	11
10–11	9	0

[a] A portion of these figures is used through the courtesy of Dr. E. E. Honey, Emergency Plant Disease Prevention Project.
[b] Diameter at breast height or 4.5 feet.

There was a high prevalence of disease on trees that were less than 8 inches d.b.h. (Table 2). The factors that governed disease prevalence on different sites probably also accounted for this fact. On the larger trees cankers were usually restricted to the branches or to the small upper part of the trunk, which is commercially unimportant.

HOSTS INFECTED, THEIR RANGE, AND CANKER DISTRIBUTION

The same Hypoxylon canker is found most frequently on *Populus tremuloides* and *P. grandidentata*, but it occurs to a lesser extent on *P. balsamifera*. Other species of poplar are also attacked by different species of *Hypoxylon*.

P. nigra var. *italica* Du Roi is attacked by *H. malleolus* B. and Rav. and *P. trichocarpa* T. and G. by *H. serpens* (P.) ex Fr. (14).

The species of *Hypoxylon* are not restricted to poplar, however. While the complete host range has not been established, *H. pruinatum* has been found producing injury on *Betula; H. morsei* on *Alnus;* and *H. blakei* on species of *Acer, Quercus,* and *Salix* (1). The work to date (1) indicates that one or another species of *Hypoxylon* may be found on most of the hardwood species.

The natural distribution of *Populus tremuloides* and *P. grandidentata,* the most frequently involved hardwoods, is reported by Sargent (12) and Munns (9). The Hypoxylon canker has not yet been reported as occurring throughout the complete host range. Its wide distribution has been recorded by several workers (1, 3, 4, 6, 8, 10). Wherever it was found, the disease had similar characteristics.

SYMPTOMS

Poplar stands infected with Hypoxylon canker stood out in marked contrast to healthy stands. Characteristic features observed were the broken trees, the dead tree stubs, and the blackened areas on the green trunks (Fig. 1, A). Cankers that girdled branches produced brown "flags" throughout the stand. If the branches beyond the canker were not dead, the leaves were usually smaller and pale green to yellow.

Incipient cankers appeared as yellow to orange, slightly sunken areas in the bark. The margins were usually irregular and lobed. On older infections blister-like conidial fructifications and erumpent perithecial stromata were clearly visible.

THE CAUSAL ORGANISM

Morphology

The morphology of *Hypoxylon pruinatum* has been discussed by Bier (1), so the details are omitted in this paper. A stroma of the imperfect stage is shown in figure 1, B, and of the perfect stage in figure 1, C. Mycelial fans and host discoloration are shown in figure 1, D. Asci, ascus stalks, and ascospores are illustrated by Bier (1).

Separate measurements were made of four perithecial collections from Madison and four from Trout Lake, Wisconsin, to determine the average size of the spore-bearing part of the ascus, of the ascus stalk, and of the ascospores. Measurements of perithecial contents mounted in water were taken with an ocular micrometer on 50 spores and 25 asci from each collection. The results, averaged by localities, are in table 3.

The spore measurements for the 8 collections agree closely with those made by Bier (1). There are some deviations in ascus measurements that may be explained by differences in the development of the asci.

The attendant taxonomy has been discussed by Bier (1). It has been confirmed and has been accepted by the present writer. There was no

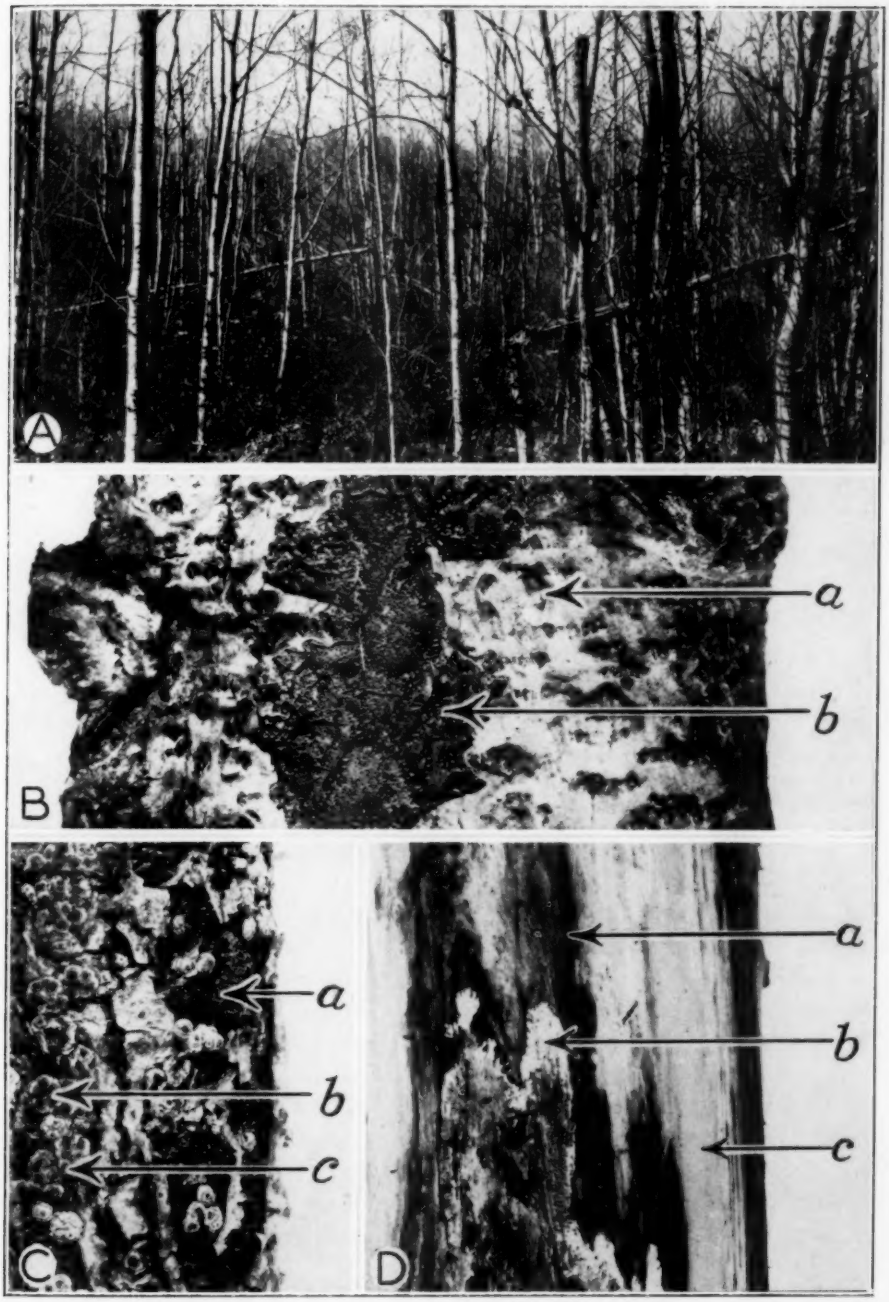

FIG. 1. A. Cankered poplar stand in Lincoln County, Wisconsin. B. Conidial stroma showing (a) "blister" of the outer bark and (b) "mycelial pegs" where outer bark has been peeled away. C. Fruiting canker showing (a) old conidial stroma, (b) perithecial stroma, and (c) ostioles of perithecia. D. Portion of canker with the bark removed to show (a) discoloration preceding mycelium, (b) mycelial fan, and (c) healthy wood.

significant change in morphology when the fungus was grown under several different circumstances.

Influence of Temperature, pH, and Certain Nutrients on Fungus Growth

The influence of temperature was investigated on the rate of growth of *Hypoxylon pruinatum* in culture on 2 per cent malt agar. Petri dishes were poured with 20 cc. each of medium adjusted to pH 6.5. Each plate was seeded with a 6-mm. disk of inoculum from a 10-day-old culture on 2 per cent malt agar. This 6 mm. was subtracted from the final growth readings. Five replications were incubated at 9 temperature levels in 4° C. steps from 4° to 36° C. Diameters were measured every other day for 8 days.

The results were averaged for 3 trials each with 5 replications on 4 single-spore isolates. The most rapid diameter growth was at 28° C. There

TABLE 3.—*Comparison of ascus and ascospore size of two Wisconsin collections and one Canadian collection*

	Material collected near		
	Madison, Wisconsin[a]	Trout Lake, Wisconsin[a]	Ottawa, Ontario[b]
	Microns	*Microns*	*Microns*
Spore-bearing part of ascus	128 to 176	152 to 187	160 to 190
Ascus stalks	32 to 80	32 to 48	40 to 60
Ascospores	22 to 29 by 8 to 13	22 to 29 by 8 to 13	24 to 30 by 9 to 13
Ascospores, average	26 by 10	25 by 10	26 by 11

[a] Collected and measured by the writer.
[b] Collected and measured by J. E. Bier (1).

was no growth in 8 days at 4°, 8°, or 36° C. The data are omitted because they are so similar to those based on weight.

Temperature also influenced the weight of mycelium produced by *H. pruinatum* in 2 per cent malt extract in distilled water. The reaction of the medium was adjusted to pH 6.5. Erlenmeyer flasks of 250 cc. capacity, each containing 150 cc. of the sterile, adjusted medium, were each seeded with a 6-mm. disk of inoculum. Five replications were then placed for 16 days in incubators at temperatures from 4° to 36° C. At the end of 16 days the mycelial mats which developed in the flasks were separated from the culture medium and were washed, dried, and weighed. The weights for 3 trials of 5 replications each were recorded and averaged for each temperature group (Fig. 2).

The greatest weight of mycelium of *H. pruinatum* was developed at 28° C. There was good growth in the range from 20° to 28° C. and no growth at 4°, 8°, or 36° C.

The importance of acidity (pH) also received attention in relation to the weight of mycelium produced by *H. pruinatum*. Malt-extract liquid

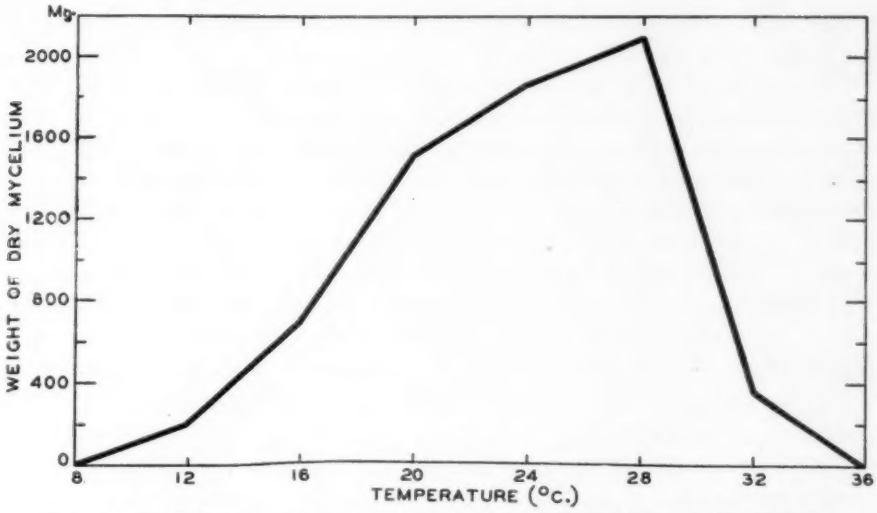

FIG. 2. The influence of temperature on growth of *H. pruinatum* in liquid culture.

medium in 250-cc. Erlenmeyer flasks was buffered with K_2HPO_4 (5 g. per liter in the alkaline range) and KH_2PO_4 (5 g. per liter in the acid range). The reaction of the medium was adjusted aseptically before inoculation according to the intervals shown in figure 3. There were 5 replications at each point of adjustment. After 16 days' incubation at 28° C. the mycelial mats were washed, dried, and weighed. The pH, read at the start and at the end of the trial, did not drift more than 0.1 pH unit in any flask during the 16 days.

The weights were averaged by pH groups. The average results of a typical trial of 5 replications are in figure 3. The greatest mycelial weight

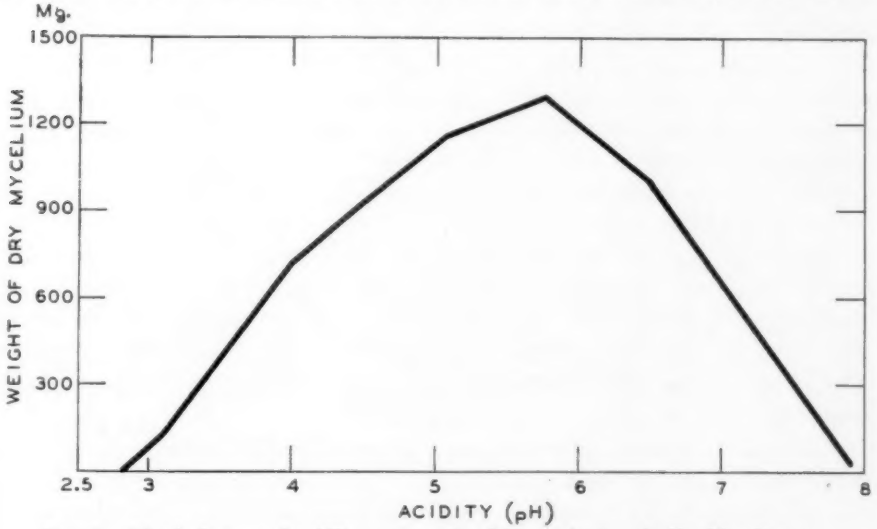

FIG. 3. The influence of acidity on growth of *H. pruinatum* in liquid culture.

of *H. pruinatum* was produced at pH 5.6. Satisfactory growth developed between pH 4.5 and 6.5.

The effect of 8 various culture media on rate of diameter growth and rate of conidial production appeared in 4 trials of 4 replications each. Six of the media used were malt agar, potato-dextrose agar, onion agar, Lima-bean agar, pea agar, and water agar. All were made according to Riker and Riker (11) and were adjusted to pH 6.5. Healthy poplar bark was ground in a Wiley Mill No. 1, was cooked in distilled water (50 g. of tissue per liter) for 1 hour, and made into 2 per cent agar. Half of the medium was made with strained tissue decoction and half with unstrained. The reaction was adjusted to pH 6.5. The same procedure was followed with Hypoxylon-infected poplar bark. Petri dishes were poured, seeded, and incubated at 28° C. for 21 days. Records on diameter growth and production of conidia were taken every other day.

The averages of 4 trials of 4 replications each showed: (1) that the cultures grew most rapidly on malt agar, filling the dishes (92 mm.) in 10 days, and slowest on cankered bark decoction agar; (2) that those on unstrained healthy bark decoction filled the dishes in 17 days; (3) that conidia were produced by cultures on malt agar in 5 days, on onion agar in 11 days, and on potato-dextrose agar in 13 days; and (4) that no conidia were produced on the other substrata within 21 days.

The investigations of the fungus as it developed *in vitro* were continued with an examination of its host relations.

ENTRANCE OF THE FUNGUS INTO THE HOST

Field inoculation studies were conducted during the summers of 1942 and 1943 to determine the means by which *Hypoxylon pruinatum* entered the host. The following types of inoculum were used: (1) mycelial cultures on 2 per cent malt agar, (2) conidial suspensions in distilled water, (3) ascospore suspensions in distilled water, and (4) ground-up masses of diseased tissue, fruiting structures, conidia, and ascospores. The conidia, ascospores, and diseased tissue were all collected from natural infections in the field.

Several types of inoculation were tried. In all cases, the tissue to be treated was first wiped over with a cloth dampened with 70 per cent alcohol and allowed to dry.

Unwounded tissue was exposed by the application (1) of 1 cm. square blocks of culture and agar, (2) of sprayed spore suspensions, and (3) of diseased-tissue inoculum. Regardless of the type of inoculum used, half of the treatments were left uncovered. One-fourth were sealed with adhesive tape, and one-fourth were wrapped with wet cotton and wax paper as described by Bier (1).

Cuts for wound inoculations were made in several ways. Cuts in an inverted "V" or "U" shape 2 to 3 cm. high by 1 to 2 cm. wide were frequently used. Small bark slits 3 to 7 cm. long, as well as bark punctures, were also used. Different wounds, respectively, were covered or left uncovered.

Some tissue was bruised and wounded before inoculation. Trunk tissue, approximately 25 to 50 sq. cm., was crushed by striking it several times with the blunt end of a hand ax. Two types of inoculation wounds were used: (1) a horizontal cut made through the bark and into the cambium near the center of the bruised area with the sharp edge of the ax, and (2) a puncture 4 to 6 mm. in diameter through the bark at the center of the bruised area. Either spore suspensions or ground-up diseased tissues were used for inoculum.

Insect borer holes were inoculated by packing into them a paste made of ground-up diseased tissue and spores in water. Half the holes were left open and half were covered with wet cloth.

Buds, leaves, petioles, and leaf axils were inoculated in both wounded and unwounded condition respectively with spore suspensions and mycelial cultures. Wounds were made with a dagger-scalpel. No attempt was made to surface sterilize the buds, leaves, petioles, or leaf axils. Spore suspensions were sprayed on, and the mycelial cultures were applied in 5-mm.-square blocks of culture and agar.

The results of the inoculations are summarized in table 4. All inoculations and controls on buds, leaves, petioles, and leaf axils, whether wounded or not, gave negative results. All inoculations and controls on unwounded branch and trunk tissues remained uninfected, as found by Bier (1). Inoculations and controls on branch stubs and in insect borer holes also remained uninfected, but it is possible that the infection court had been closed by wound tissue before inoculation. The inoculations on cut branch and trunk tissue were from 4 to 60 per cent positive. All covered controls were uninfected, but 7 per cent of the open controls became infected. The infection of the open controls was doubtless caused by natural disease spread from fruiting cankers in the vicinity. All inoculations on bruised tissue were positive. All covered controls remained uninfected. The open controls were 60 and 50 per cent infected, respectively, for ax wounds and for puncture wounds on bruised areas. Natural spread from fruiting cankers was again held responsible. The data indicate that *H. pruinatum* is a wound parasite on bark tissue.

Several types of branch and trunk wounds were found during field observations in Wisconsin during the summers of 1941, 1942, and 1943 that could act as infection courts in natural poplar stands. Some of these infection courts, all of which had been found at one time or another at the centers of cankers, were caused (1) by weather, *e.g.*, wind, ice, lightning, (2) by man, *e.g.*, ax wounds, logging damage, (3) by animals and birds, *e.g.*, deer scraping, porcupine chewings, sap-sucker borings, and (4) by insects, *e.g.*, bark borers, chiefly *Saperda calcarata* Say. A supplementary study was made on the relation of insect-borer holes to incidence of new cankers.

All of the insect-borer holes up to 8 feet above the ground were examined in all the poplars on 11 plots, each covering $\frac{1}{10}$ acre, located in Dane, Vilas, and Waushara Counties in Wisconsin. Of the 1018 borer holes examined,

TABLE 4.—*Results of the 1942 and 1943 field inoculations with Hypoxylon pruinatum*

Kind and place of inoculation	Kind of inoculum	Covered or open	Treatments	Hypoxylon cankers
			Number	*Per cent*
Young growth				
Buds, leaves, petioles, and leaf axils; wounded and not wounded	conidial and ascosporic suspensions, mycelial cultures, controls	half of each covered, rest open	1600	0
Older growth				
Branch and trunk				
Unwounded	do plus mass inoculum	do	200	0
Wounded				
Slit, inverted "U" or "V" cut	conidial suspensions	covered	40	37
		open	40	31
	ascosporic suspensions	covered	40	50
		open	40	60
	mycelial culture	covered	40	46
		open	130	37
	control	covered	120	0
		open	210	7
Bark puncture	mass inoculum	covered	25	4
	control	covered	25	0
Bruised and ax cut	mass inoculum	covered	40	100
		open	40	100
	control	covered	40	0
		open	40	60
Bruised and puncture	mass inoculum	covered	20	100
		open	20	100
	control	covered	20	0
		open	20	50
Insect borer holes	mass inoculum and controls	half of each covered, rest open	100	0
Dead branch stubs	mass inoculum	open	25	0
	control	open	25	0

14, or 1.4 per cent, were at the center of cankers. Since so few of the borer holes were infected, the borer itself did not seem to be an active agent in the dissemination of the disease. However, it was obvious that the borer holes were open infection courts and, because of their great numbers in the woods, had to be considered in the infection complex.

From insect injuries and other wounds a dark, sticky liquid frequently was exuded, which, during dry weather, hardened into a "varnish." Some of this exudate was incorporated into water-agar medium and seeded with a mycelial culture of *Hypoxylon pruinatum*. Water-agar controls were used. Two trials of 5 replications each were incubated at 28° C. for 16 days. The resulting cultures on water-agar plus "varnish" had an average diameter that was 40 per cent greater than that in the controls. This suggests that the sticky wound exudate might act not only as a spore catcher, but also as a

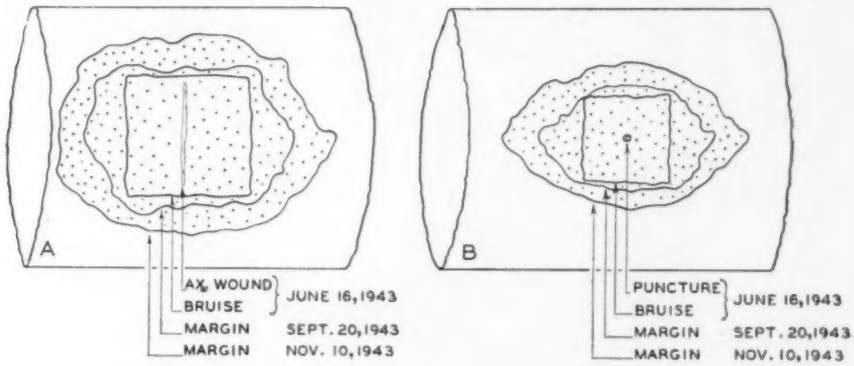

FIG. 4. Average advance of artificially induced cankers. A. Square bruised area with an ax wound, average of 24 cankers. B. Square bruised area with a puncture wound, average of 17 cankers.

culture medium when suitably wet. The entire insect relations problem deserves further study.

The development of lesions following inoculation was recorded for 200 different trees. The first symptoms appeared in 2 to 8 weeks. The rate of canker spread, which was most rapid up and down the tree, was observed on 41 cankers from 2 types of wounds shown diagrammatically in figure 4. The measurements were based on the surface appearance and independent from the spread of the fungus within the tissues.

DEPTH OF FUNGUS PENETRATION

When the bark was removed from active Hypoxylon cankers, mycelial fans frequently were found growing over the surface of the wood (Fig. 1, D). To determine how deeply the fungus penetrated into the host tissues, radial sections of active cankers were made on a sliding microtome. A differential stain was secured with the methyl violet–Bismark brown technique described by Hubert (5). Microscopic examination of the sections showed mycelium in the bark, the cambium, and the outer 4 to 5 mm. of wood tissue. These

observations were checked by cultural studies, in which cankered tissues outside of the cambium were removed with sterile scalpels to prevent contamination of the inner tissues. Chips of wood were taken from different depths in the wood tissue and were transferred aseptically to 2 per cent malt agar in Petri dishes. Isolations were made also from the bark and the cambium. Incubation was at 28° C. for a maximum of 16 days. The data (Table 5) indicate that the organism is located in the host bark, cambium, and outer 8 to 9 mm. of the wood, where it apparently had an opportunity to live from one season to another.

TABLE 5.—*Results of attempts to isolate Hypoxylon pruinatum from different depths in host tissue (Populus tremuloides)*

Tissue and depth cultured	Specimens	Isolations	Isolations yielding Hypoxylon
	Number	*Number*	*Per cent*
Bark	1	48	100
Cambium	1	48	100
Wood			
0.5 mm. below cambium	1	48	100
1.0 do	2	96	100
3.0 do	2	96	75
5.0 do	4	192	12
8.0 do	2	100	2
10.0 do	2	100	0

OVERWINTERING

The means of overwintering of the fungus was determined by isolation of 32 single ascospore and 50 mass-tissue cultures from 5 cankers that were collected every month from November to April. At least 60 per cent of the ascospores were viable, and 80 per cent of the tissue isolations yielded *Hypoxylon* every month. No viable conidia were found during this period. This indicated that the organism overwintered both as mycelium in infected tissue and as ascospores which were already available to spread the fungus the following season.

DISSEMINATION

The natural means of dissemination of *Hypoxylon pruinatum* was studied during the summers of 1942 and 1943. To determine the direction of natural spread in the field, the locations were plotted of all new and old infections on 4 one-tenth acre plots, 2 in Vilas County and 2 in Dane County, Wisconsin. The prevailing summer winds for these two areas were from the south and the southwest.[4] Of the 250 new cankers examined, 80 per cent were down wind from and facing toward the old fruiting infections. This observation prompted experimentation on wind dissemination.

A spore trap was constructed that was similar to Maddox's "Aeroconiscope" (2). The trap was set up in the center of a poplar plot 15 feet from the nearest canker. The spore content of the trap was examined every week

[4] Courtesy of Eric Miller, U. S. Weather Bureau, Madison, Wisconsin.

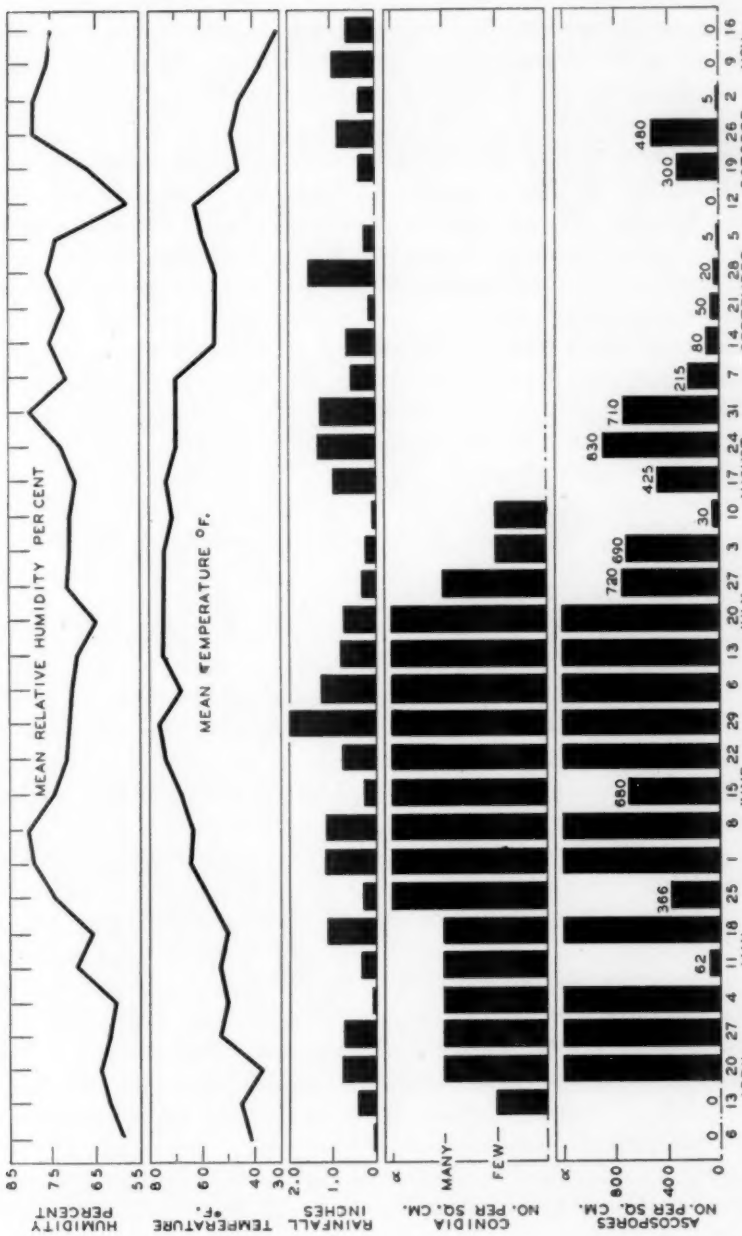

FIG. 5. Mean relative humidity, mean temperature, rainfall, and spore trap counts in Hypoxylon canker study plot, Madison, Wisconsin, 1943.

from April to the middle of November. The results of the counts and the accompanying weather records are in figure 5. Conidia or ascospores, or both, were present in the air from the second week in April through October, with the exception of the second week in October when there was no rain. This indicates that wind dissemination was in progress throughout the entire period.

Further evidence on wind dissemination came from infection counts on trees that were bruised and wounded (see table 4). Sixty wounds were covered with adhesive tape and 60 were left open. By the end of 5 months, 57 per cent of the open wounds were infected with *H. pruinatum,* while all the covered wounds were uninfected. This could be explained best on the basis of wind-borne spores.

An attempt was made to study the natural incidence of disease in relation to time of year, meteorological conditions, and availability of inoculum. However, the incidence of disease each week, which was less than half of one per cent, gave figures that were too small for satisfaction. To secure significant figures would have involved frequent observations on thousands of tagged trees. Since it was not feasible to examine such numbers, attention was directed toward the influence of weather on the availability of inoculum.

ENVIRONMENTAL CONDITIONS INFLUENCING CONIDIAL DETACHMENT AND ASCOSPORE DISCHARGE

The influence of time of year and of environmental conditions favoring and retarding conidial detachment and ascospore discharge was investigated during the summers of 1942 and 1943. Greased microscope-slide spore traps similar to those described by Bier (1) were placed on cankered trees so that the incidence and intensity of spore release could be observed. The greased slides over 4 cankers showing the perfect stage of *Hypoxylon pruinatum* were placed 4 mm. away from the perithecial stromata. Those on the 2 conidial cankers were placed facing the prevaling winds. Spore counts were made on all the slides every week from April to the middle of November. The number of ascospores was counted up to 900 spores per sq. cm. but beyond that was given an infinity notation. The conidia were small and hyaline, so their number was estimated. The value "Few" equalled 0 to approximately 100, "Many" equalled approximately 100 to 500, and "∞" equalled over 500 spores per sq. cm. The count of the spore load in the "Aeroconiscope" spore trap is in figure 5.

Conidia first appeared in the trap the second week in April. The load increased to a maximum by the fourth week in May and held a constant high level until the third week in July when it started to drop off to an end the third week in August.

A comparison of the trap record with temperatures and humidities for the period suggests that the increase from April to June in the numbers of conidia dispersed was correlated with the increase in mean temperatures and

mean relative humidities during the same period. There appeared to be no correlation with rainfall.

To determine whether free water was necessary to conidial detachment, greased glass slides were fastened 1 cm. away from 5 cankers showing the imperfect stage. Conidial counts were made on the slides for 10 one-hour periods during 10 days that were rainless. This was repeated for 10 rainy days. A representative average showed that 234 conidia and 182 conidia were caught per hour per sq. cm. of glass slide during dry periods and rainy periods, respectively. The trend of these observations was confirmed repeatedly under controlled laboratory conditions. This indicated that free water was not necessary for conidial detachment.

The first ascospores were found in the trap during the third week in April and the last ones were found on the second of November. The two end points seem to be correlated with a mean temperature below 45° F. The low mean temperature for the week ending April 20 was caused by two cold days, April 14 and 15. The heavy spore discharge for that week took place on April 17, 18, and 19. The high mean summer temperatures did not seem to influence ascospore discharge. The mean relative humidity, except when it indicated the absence of rain, *e.g.*, week ending October 12, had no influence on the incidence of ascospore discharge. There was a rather close correlation between the amount of rain and the volume of spore discharge.

The ascospore release was recorded at 2-hour intervals in the field from 10 p.m. June 14 to 10 a.m. June 16, 1943. Spore counts were made on 4 greased glass slide traps. The average results of the spore counts and the corresponding weather data are in figure 6.

There was no significant relation between temperature and amount of ascospore discharge for the 36 hours of the trial. There was an apparent relation between relative humidity and ascospore discharge. Closer examination of the data reveals, however, that the periods of rain, which influenced the relative humidity, were more likely the critical factors in spore discharge. This is illustrated by the records taken at 6 a.m. June 15. At that point, between rains, the relative humidity was still approximately 100 per cent, but the spore discharge had fallen to 700 spores per sq. cm. The spore discharge fell to 50 spores per sq. cm. at 10 a.m., approximately 2 hours after the 6:10 to 7:30 shower, even though the relative humidity was still approximately 100 per cent in the woods. This suggests that free water is necessary for the initiation of ascospore discharge.

A few ascospores were caught in the traps as long as 20 hours after a rain. It may be that ascospores caught in drops of water during the rain clung to the tree and were blown off long after cessation of ascospore discharge from the perithecia; that ascospores that had fallen to the ground were redistributed by air currents; or that ascospores floating in the air for a long time finally came to rest on the slides.

The necessity of free water in initiating ascospore discharge was studied in the laboratory. A chamber (Fig. 7) was constructed in which the relative

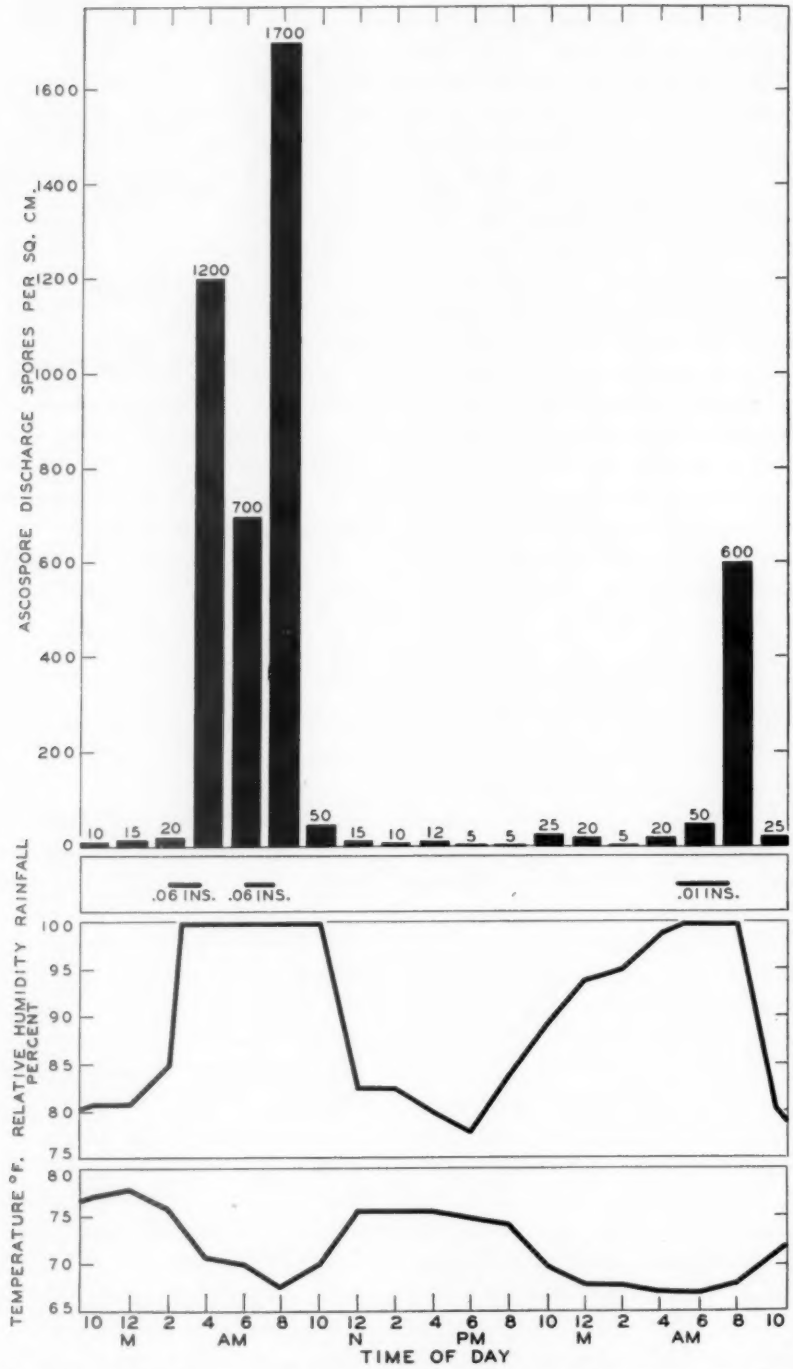

FIG. 6. Temperature, rainfall, relative humidity, and ascospore discharge in Hypoxylon canker study plot, Madison, Wisconsin, June 14 to 16, 1943.

humidity was regulated by a stream of water-saturated air. Water was atomized into a 5-gal. carboy. The water-laden air from there was run through a water trap to remove any free moisture and from there into the chamber. The relative humidity in the chamber was calculated from wet and dry bulb thermometer readings.

Air-dry perithecial stromata were placed in the chamber and kept for 21 days in an atmosphere without free water. No ascospores were discharged. The relative humidity approached saturation, and so was comparable to that in the woods during a rain. This was repeated 3 times each with different perithecial stromata but with the same result. When perithecial stromata from the same source were soaked in water for one-half hour before being placed in the chamber, ascospore discharge was recorded as early as one hour after introduction into the chamber and averaged 407

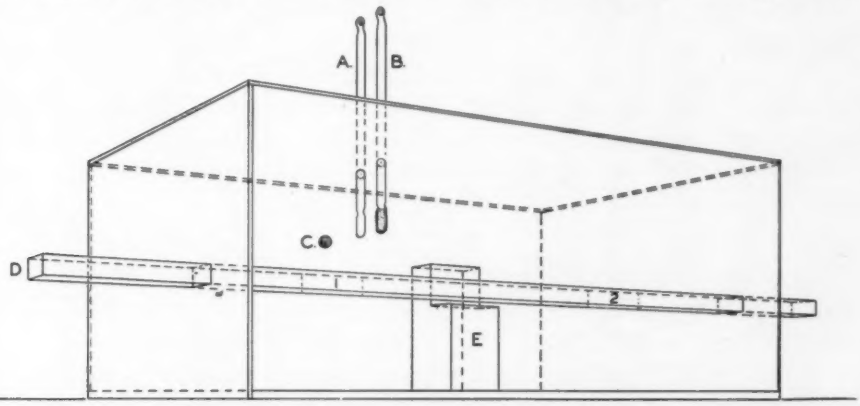

FIG. 7. Ascospore discharge chamber with front face removed. A and B. Dry and wet bulb thermometers. C. Inlet hole for water-saturated air. D. Sliding bar carrying greased glass slides 1 and 2 in such a way that slides could be removed and replaced without opening chamber. E. Standard on which perithecial stromata were fastened so as to face the greased slide and allow 4 mm. between ostioles and slide.

spores per sq. cm. per hour for a 3-hour period in a relative humidity approaching saturation. Thus, free water was necessary for the initiation of ascospore discharge.

DISCUSSION

Various lines of work, reported by earlier writers (1, 6, 8, 10) about Hypoxylon canker on poplar, have been repeated and confirmed for Wisconsin, although the details have been largely omitted to save space. Among these items are the following: the economic importance of poplars, the destructiveness of Hypoxylon canker, and the morphology and attendant taxonomy of the causal organism.

The means by which this fungus operates both in the laboratory and in the woods have been elucidated. The study of the life history in relation to pathogenesis has shown how the fungus grows and sporulates at different times of year and under varying conditions of weather. The relatively low

percentages of positive results suggest that this fungus infects with some difficulty through wounds made by cutting. However, if it can start in association with bruised and killed tissue, infection occurs easily and practically every time. A practical consideration is the development of an inoculation technique whereby one can be confident of a high percentage of infection from artificial introduction of spores. This is a critical item for making quality tests of poplar selections.

The development of rapidly growing poplar hybrids, which are capable of rooting from cuttings, has attracted considerable attention in recent years. However, if such hybrids should prove as susceptible to Hypoxylon canker as many of the native poplars, plantations of them will certainly lead to serious disappointment. By means of the technique now made available, tests of resistance by these and other selections to Hypoxylon canker can be employed with confidence, and the susceptible selections can be eliminated.

Meantime, from the several lines of evidence presented, it is clear that Hypoxylon cankers in the woods are a continual source of inoculum for any healthy trees in the neighborhood. Consequently, if the value of the stand justifies the expense, the trees affected with Hypoxylon canker should be removed and burned.

SUMMARY

A study was made of *Hypoxylon pruinatum*, of its life history in relation to pathogenesis on poplar, and of the associated Hypoxylon canker which is the most important disease on poplar in the Lake States.

The Hypoxylon canker was found on an average of 24 per cent of all the poplars examined in Wisconsin and the corresponding symptoms are described.

Surveys showed that there was more canker on poor growing sites than on good sites.

The causal organism made the best diameter growth in culture and developed the greatest mass of mycelium at 28° C. The optimum hydrogen-ion concentration range for growth in culture was from pH 4.5 to 6.5. The fastest rate of growth and rate of conidial production observed was on 2 per cent malt agar.

The organism is a wound parasite on branches and trunks, but buds, leaves, petioles, and leaf axils were not infected. Only a moderate percentage of infection developed from inoculation wounds made by cutting. However, one hundred per cent infection was secured when the trunk and branch bark was bruised and wounded before inoculation. Examination of the cankers revealed the organism in the bark, cambium, and no deeper than 8 mm. into the wood.

The organism overwintered in host tissue as mycelium and ascospores and was disseminated by wind.

Conidia were detached under either wet or dry conditions, but free water was necessary for initiating ascospore discharge. There was no ascospore

discharge in the early spring or the late fall when the mean temperature was below 45° F.

By means of inoculations through bruised tissue a method has been developed for testing the relative resistance of various poplar selections.

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